Quantifying individual variation in fine-scale time and energy trade-offs in breeding grey seals: How do differing behavioural types solve these trade-offs?

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Quantifying individual variation in fine-scale time and energy trade-offs in breeding grey seals: How do differing behavioural types solve these trade-offs?

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Abstract

Lactation is one of the most energetically demanding periods of any female mammal's life history, where individuals strike a balance between limited resources between their daily activity and towards the growth of their offspring, while still maintaining enough energy stores to maintain themselves in the process. Capital breeding systems mean that females must sustain themselves and their offspring while fasting exclusively on energy reserves acquired beforehand. Female phocids as a result must deal with pressures of a brief terrestrial existence where trade-offs in time, behaviour, energy, and responsiveness to the environment can have tangible consequences to short-term fitness and health.

The aim of this thesis was to use new techniques, specifically animal-borne accelerometers and heart rate monitors, to track behaviour and physiology and assess the inherent trade-offs therein through the core duration of lactation in a capital breeding phocid, the grey seal (*Halichoerus grypus*). Female grey seals were equipped with biologging devices on the Isle of May over three consecutive breeding seasons. Using accelerometry and heart rate techniques, I aimed (1) to remotely classify behaviour using machine learning techniques, (2) to assess trade-offs in time-activity for the duration of lactation, (3) to build a holistic picture of energy allocation within the species, and (4) to develop new methods for tracking heart rate and breathing for terrestrial mammals using grey seals as a model. I also assessed the effect that consistent individual variability in behaviour, stress-coping styles, may have on the methods developed here and how they may drive behaviour and energy trade-offs over time.

Accelerometers presented a useful way to remotely track several key behaviours, accurately classifying the core static behaviours over lactation. Consistent individual differences in stress-coping styles, as determined from measures of heart rate variability, modulated almost every aspect of behaviour and physiology measured in this study. More specifically, consistent trade-offs were identified for grey seal mothers between balancing time spent in a state of rest against remaining vigilant across multiple contexts, but also that these individual differences drove how individuals manage and expend that energy, ultimately resulting in differences in short-term fitness outcomes. Effort towards nursing, however, appeared to be largely fixed. Individual differences in energy management also appear to result in different levels of plasticity to environmental pressures, suggesting that future ambient conditions may not be suitable for breeding seals. This thesis also successfully detected breathing rates on land, revealing new evidence as to the energy saving and water conservation benefits of regularly engaging in periods of breath-hold while at rest.

Overall, this thesis has provided new tools for exploring behaviour and physiology, and the inherent trade-offs therein, with minimal disturbance to lactating phocid seals. These differences, while minute in the scope of evolutionary constraints, may be among the most important drivers for the success and survival of populations in the face of greater environmental variability as the climate continues to change.

Declaration

The material contained within this thesis has not been previously submitted for a degree at Durham University or any other university. The research reported here has been conducted by the author unless otherwise stated. All previous work has been appropriately documented.

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List of Abbreviations

Abbreviation: Definition, **Equation number** (first page used/defined)

ACC: Accelerometer data logger, AXY-Depth or AXY-Trek models (29)

adj-*R*: Bootstrapped repeatability, adjusted for fixed effects in model (63)

AEE: Activity-specific energy expenditure (47)

AICc: Akaike information criterion, corrected for small sample size (102)

aux-f_H: Daily auxiliary heart rate, proxy for activity-specific energy expenditure (48)

BCS: Behavioural classification scheme, used to define broad behavioural states at binomial -'01', coarse -'coa', medium -'med', fine -'fine', and context-specific resolutions -'con' (38)

ColLoc: Location on Isle of May colony, defined by 7 geographically distinct locations (106)

DEE: Daily energy expenditure (47)

DMT: Daily maximum temperature, ^{o}C (99)

dn: Binomial dummy variable for day, 0, vs. night, 1, split by civil twilight (100)

dy: Dynamic acceleration, static acceleration subtracted from raw acceleration in each axis X, Y, or Z (46)

ECG: Electrocardiography, measure of electrical impulse associated with heart beats (28)

F1: Harmonic mean of precision and recall, **Eq. 3-6** (62)

FDR: False discovery rate, proportion of incorrectly detected events of total, **Eq. 6-2** (179)

fft: Fast Fourier transform (43)

FIR: Finite impulse response, processing filter for periodically sampled signal (175)

Freq: Frequency corresponding to the primary, 1, or secondary, 2, power spectrum density in each axis X, Y, or Z (46)

f_s: Sampling frequency (43)

GLMM: Generalized linear mixed-effects models (96)

GPS: Global positioning systems (25)

HRM: Heart rate monitor unit, Firstbeat Technologies (174)

HRV: Heart rate variability (93)

IBI: Inter-beat intervals, time lag between successive heart beats (28)

ID: Unique individual alphanumeric identifier (24)

jerk: Derivative of acceleration in each axis X, Y, or Z, or as the norm N, **Eq. 2-3** (43)

kNN: *k*-Nearest Neighbours, machine learning algorithm (57)

Lphase: Phase of lactation, binned into 6 day intervals (99)

LRT: Likelihood ratio test (104)

mdml: Maternal daily mass loss, mass-specific rate over lactation, in g kg^{-1} day^{-1} (47)

mean-f_H: Mean daily heart rate, proxy for daily energy expenditure (48)

mean.LFHF: Median ratio of low frequency to high frequency elements of heart rate (100)

mean.PNSI: Mean parasympathetic nervous system indicator, ratio of high frequency components to total signal power in heart rate frequency (99)

MEO: Milk energy outputs (153)

min-f_H: Daily minimum heart rate, proxy for metabolism and background energy use (48)

MPPM: Maternal post-partum mass, kg, estimated based on mass loss over lactation (47)

MT_{eff}: Mass-transfer efficiency, ratio of maternal mass loss to pup mass gain, %, **Eq. 2-4** (47)

Mwind: Mean daily wind speed, kilometres per hour (99)

ODBA: Overall dynamic body acceleration, **Eq. 2-1** (6)

OOB: Out-of-bag error estimates, bootstrapped data used to build classification trees within a random forest algorithm (61)

P: Precision, proportion of correct positive behavioural classifications, **Eq. 3-4** (62)

PCV: Packed cell volume, %, approximate proportion of red blood cells to whole blood volume, measured in microcapillary tubes (181)

PDBA: Partial dynamic body acceleration, absolute dynamic acceleration in each axis (42)

PPV: Positive predictive value, analogous to precision (see also *P*) in event detection algorithms (179)

Precip: Total daily precipitation, mm (99)

PSD: Power spectrum density, power of signal in frequency domain in decibels (42)

R: Recall, proportion of correctly classified new data, **Eq. 3-5** (62)

rMSSD: Root mean square of successive differences in inter-beat intervals (99)

SD1:SD2: Ratio of median value for two dimensional ellipse of heart rate frequency distribution (100)

st: Static acceleration, derived as running mean over 3 s, in each axis, X, Y, or Z (46)

T_{uc}: Upper critical temperature, defines upper ambient temperature boundary of thermoneutral zone for an organism (214)

TPR: True positive rate, analogous to recall (see also *R*) in event detection algorithms (179)

T_{lc}: Lower critical temperature, defines lower ambient temperature boundary of thermoneutral zone for an organism (214)

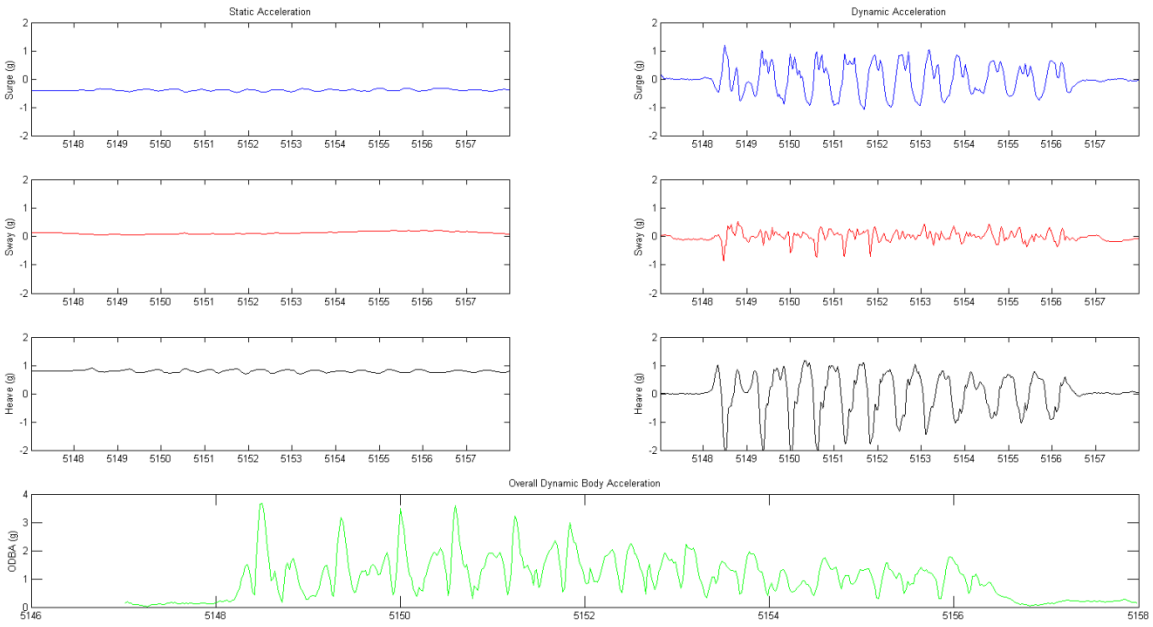
VeDBA: Vectorial dynamic body acceleration, **Eq. 2-2** (6)

VeDBAs: Smoothed vector of vectorial dynamic body acceleration, calculated over 3 s (43)

VO_2: Volume of oxygen consumed per litre of air (6)

Chapter 1:

Introduction



1.1 – Behavioural observation

The collection of behavioural observations has been the foundational methodology of ethological studies. First formalized in Altman (1974), observational efforts were given a standardized framework to develop a quantitative means of describing behaviour. Observational studies are the foundation of traditional studies of behaviour and allow for the dissection of life-history trade-offs and the subsequent fitness consequences associated with the apparent selection pressures of the environment. Behavioural observations in the field have been used successfully to investigate a broad range of research questions including aspects of maternal investment (Anderson and Fedak 1985, 1987; Trillmich 1990; Loughry 1993; Boness and Bowen 1996; Hood and Ono 1997; McLean and Speakman 1999; Maniscalco et al. 2006; Maniscalco 2014) and reproductive success (Caro 1987; Tinker et al. 1995; Van Parijs et al. 2003; Dingemanse et al. 2004; McGhee and Travis 2010; Twiss et al. 2012a; Yoshida et al. 2016). Observational studies have also been employed to examine individual- and population-level trade-offs associated with the behavioural aspects of foraging (Le Boeuf et al. 1996; Burns 1999; Beck et al. 2003; Fossette et al. 2010; Horning 2012), as well as energetics (Brodie and Paasche 1985; Costa et al. 1986; Lydersen and Kovacs 1999; Harding et al. 2005; Yeates et al. 2007; Witter et al. 2012; Mosser et al. 2014). Observational studies employ various sampling regimes including instantaneous or scan sampling, focal animal sampling, or all-occurrence sampling to construct metrics like time-activity budgets or relationships to nearby conspecifics and dyads (e.g. Altmann 1974; Maniscalco et al. 2006; Pelletier et al. 2009; Portugal et al. 2010; Bishop et al. 2014; Yoshida et al. 2016; Závorka et al. 2016; Robinson et al. 2017). These standardized techniques attempt to capture a representative sample of daily activity or behavioural reactions to their surrounding stimuli, while minimizing disturbance as much as possible (Altmann 1974).

While behavioural observation methods have been very successful in many cases, these approaches may have limitations in their applicability. Observational approaches often require long hours in close proximity to study individuals, potentially biasing behaviour. This is especially true in observational studies of shy or cryptic species where observation time is limited by geographic location or activity patterns (Byrnes et al. 2011; Brown et al. 2013; Williams et al. 2017; Bino et al. 2018). Even those species that are observable may not always be accessible at certain life history stages. Such is the case for pinnipeds, where observation time is limited to when the animal is hauled out on land, biasing effort towards lactating adult females and territorial males, while juvenile age classes may be missed entirely (e.g. Maniscalco and Atkinson 2002; Raum-Suryan et al. 2002). In addition, many species may also lack observations outside of their peak active period, be they diurnal, nocturnal or crepuscular (Anderson 1978; Byrnes et al. 2011; Culloch et al. 2016).

To improve accessibility, some have employed remote video monitoring systems and camera traps to isolated areas with minimal disturbance to the study species (e.g. Mills et al. 2005; Hariyadi et al. 2011; Swanson et al. 2015; Weinstein 2015; Caravaggi et al. 2017). However, some researchers may be precluded from these remote video methods due to lack of sufficient funding to set-up, access, and maintain these systems. In the case of observing behaviour in the dark, some researchers have attempted to use night vision or thermal cameras to catalogue behaviour when visual observations are no longer possible (e.g. Culloch et al. 2016). While these cameras can also be prohibitively expensive, the quality of footage may prevent the identification of certain behaviours, but also may preclude the use of natural patterning or scars as unique identifiers of individuals (Maniscalco et al. 2006; Hiby et al. 2013; Paterson et al. 2013). Even when disturbance is minimized through such advanced remote-monitoring means, footage without proper contextual information, delays in recording, researcher subjectivity in behavioural definitions, and inappropriate sampling design may still

play a role in the interpretation of behaviour from observations (Altmann 1974). These limitations for both traditional *in situ* observations and newer remote monitoring approaches have a potential to contribute to significant error in generating activity budgets may belie behaviour and fitness outcomes such as reproductive success.

1.2 – Accelerometers and behaviour

Accelerometers present an emerging tool for remotely classifying behaviour using animal-mounted data loggers (Brown et al. 2013). Studies employing accelerometers are becoming more and more prominent in the literature; as a unit, accelerometers are popular not only due to their relatively small size, but also due to their flexibility in application and implementation across a wide variety of taxa (Shepard et al. 2008; Halsey et al. 2009a; Byrnes et al. 2011; Brown et al. 2013; Lyons et al. 2013). As a result of this, accelerometers have been applied to very small-bodied species such as alpine and lodgepole chipmunks (*Tamias alpinus* and *T. speciosus*, respectively; Hammond et al. 2016), slow-moving terrestrial chelonians (e.g. *Testudo graeca*; Lagarde et al. 2008), larger-bodied birds (Halsey et al. 2009a; Kays et al. 2011; Shamoun-Baranes et al. 2012), and all the way up to the largest vertebrate, blue whales (*Balaenoptera musculus*; Goldbogen et al. 2013). These new tools also appear to overcome many of the inherent problems associated with traditional behavioural observation by allowing for the collection of data without observer bias across a full 24-hour period, if needed (Fossette et al. 2010; Brown et al. 2013; Evans et al. 2013). Depending on the needs of the researcher, accelerometers can measure anywhere between one and three dimensional axes (X – 'surge'; Y – 'sway'; Z – 'heave'), and can be mounted solo or are often found in combination with a variety of other sensors like magnetometers, gyroscopes and pressure instruments, such as the so-called 'daily diary' tag (Wilson et al. 2008; Hazen et al. 2012; Evans et al. 2013).

Early trials of these methods were originally developed in a laboratory setting, investigating movement in humans and domesticated animals from a clinical perspective (Karantonis et al. 2006; Martiskainen et al. 2009; Robert et al. 2009; Hokkanen et al. 2011; Preston et al. 2012). Subsequent developments have moved out of the laboratory and have seen success in application to terrestrial and arboreal species in a wild setting (Byrnes et al. 2011; Grünewälder et al. 2012; Lush et al. 2016). Increasing miniaturization has also allowed for deployments on small-bodied and volant animals such as birds and fruit bats (Elliott et al. 2012; Fahr et al. 2015; Hammond et al. 2016). More recently, accelerometers have been used to investigate diving and foraging behaviour in aquatic species (Fossette et al. 2010; Battaile et al. 2015), such as individual prey capture events in marine mammals (Skinner et al. 2010; Ydesen et al. 2014; Owen et al. 2016), flight and diving patterns in aquatic birds (Yoda et al. 2001; Sakamoto et al. 2009) as well as remotely classifying an individual's time-activity budgets (Byrnes et al. 2011; Campbell et al. 2013; Watanabe and Takahashi 2013; Bidder et al. 2014; Graf et al. 2015; Alvarenga et al. 2016). Understandably, these devices have been particularly favoured in many species that are cryptic or impossible to access using behavioural observation approaches (Fahlman et al. 2008; Fossette et al. 2012; Ydesen et al. 2014; Battaile et al. 2015). This is especially true for cetaceans where their observable time is limited to short periods when the animals are at the surface and data on subsurface behaviour are sparse or non-existent (Goldbogen et al. 2006, 2008, 2013). In the case of rorqual whales (Family *Balaenopteridae*), suction cup-mounted accelerometers have given an unprecedented look at both the time spent foraging, the efficiency of dives calculated by the number of lunges sensed, and the kinematics of these behaviours that would be impossible to obtain through normal physiological studies (Johnson and Tyack 2003; Goldbogen et al. 2006, 2013; Owen et al. 2016). This emerging technology has made it possible to study complex behaviour without the

need for direct observation, overcoming many of the limitations that have prevented behavioural investigations in species who are out-of-sight for most of their time.

1.3 – Remote monitoring of behavioural physiology

Acceleration has also permitted an alternative way to infer energy expenditure associated with the movements and behaviours found in these time budgets by deriving Overall or Vectorial Dynamic Body Acceleration (ODBA or VeDBA, respectively; Fahlman et al. 2008; Gleiss et al. 2011; Halsey et al. 2011a; Qasem et al. 2012). These proxies are calculated by either summing the dimensional axes of acceleration or calculating the mean directional vector of an individual's movement trajectory, respectively. ODBA has been found to be a good predictor of the rate of oxygen consumption ($\dot{V}O_2$) for the cost of locomotion and other behaviours, though the relationship has only been validated for a few species (e.g. Wilson et al. 2006). While the two ways of deriving dynamic body acceleration are relatively equal in their predictive ability of energy usage rates, VeDBA has been shown to be most useful when the orientation of the logger is not known, such as is the case for many studies involving whales where the 'tag-frame' (the orientation of axes that the tag senses) does not necessarily align with the 'animal-frame' of reference (the orientation of axes that the animal moves within; Qasem et al. 2012; Goldbogen et al. 2013). Some authors have even attempted to use these relationships of $\dot{V}O_2$ and behaviour to price out individual units of movement to total energy usage over small time-scales (e.g. Williams et al. 2004). However, these relationships relating movement to discrete units of energy usage are only useful when properly calibrated to the species in question (Halsey 2017).

When combined with a measurement of metabolic rate such as heart rate as a proxy for $\dot{V}O_2$, the ability to predict energetic expenditure by incorporating apparent energetic efficiency and basal conditions becomes more accurate, but again has only been validated for a limited

number of species (Halsey et al. 2009b; Gleiss et al. 2011; Portugal et al. 2016). While the applications of ODBA and VeDBA have presented a novel way to assess the actual cost of locomotion and other behaviours in a variety of contexts, the addition of heart-rate allows for a proxy of an individual's resting metabolic rate that would be missed using acceleration only when an animal is unmoving (Butler et al. 2004). Heart rate has long been an accurate and robust measure of energy expenditure in wide variety of taxa (Heldmaier et al. 2004; Gremillet et al. 2005; Halsey et al. 2011b; Bishop and Spivey 2013; Briefer et al. 2015). Recent work has found that various metrics of heart rate have been found to be reliable indications of daily energy expenditure, metabolism, and activity-specific energy expenditure as determined from measuring $\dot{V}O_2$ for several species of birds and mammals (Portugal et al. 2016). Pinnipeds and other diving mammals undergo extreme changes in heart rate by alternating between very high tachycardia and very low bradycardia not only as a response to diving, but also appear to retain this ability to modulate heart rate on land while sleeping (Fedak et al. 1988; Castellini et al. 1994; Andrews et al. 1997; Horning 2012; Williams et al. 2017).

Deriving energy expenditure from heart rate when combined with the addition of unbiased time-activity budgets, allows for a fine-scale look at the variability within and between individual energetic expenditures. This multi-channel approach has allowed researchers to identify long-term energetic and behavioural trade-offs in breeding loggerhead sea turtles (*Caretta caretta*; Fossette et al. 2012) and novel flight strategies for overcoming one of the highest altitude migrations in the world in bar-headed geese (*Anser indicus*; Bishop et al. 2015b) that would have been unattainable through traditional observational methods nor through sole physiological methods. Very little work to date has been able to get heart rate from free-ranging pinnipeds and very few attempted to investigate the energetic trade-offs therein (e.g. Williams et al. 1991; Andrews et al. 1997; Castellini and Zenteno-Savin 1997; Green et al. 2009a; Chaise et al. 2017; Kaczmarek et al. 2018). Using new techniques from

multiple channels of behaviour and physiology, this thesis seeks to build novel and robust estimates and discussions of time and energy trade-offs in a wild pinniped.

1.4 – Ability and limitations of emerging technology

Most accelerometer work to date has only been able to resolve behavioural activity budgets with a relatively low number of coarse behavioural categories such as active, inactive, moving, or feeding. With this in mind, Graf et al. (2015) point out several trade-offs in behaviour detectability with accelerometers. Namely, that a given sampling frequency may introduce extra data, or noise, that will mask the ability to interpret transient behaviours, multiple behaviours occurring simultaneously, or distinct behaviours that may involve very little movement. In a more simplistic sense, it is not yet clear how well one can interpret meaningful behaviour from three separate axes with either too much noise (sampling frequency is too high) or too little information (sampling frequency is too low). While such tools as low-pass filters exist to sieve the larger signals out, the decisions on what signals are more important than others remains with the observer to evaluate (Brown et al. 2013). Accelerometers are also unable to interpret the environmental context and how that may be affecting an animal's physical orientation or dictating behaviour, such as would be experienced by a diving mammal that is using passive buoyancy to move rather than activity (e.g. (Davis and Weihs 2007), as these tags remain effectively blind to their surroundings outside of any other sensors that may be included (Shepard et al. 2008). It is also important to note that accelerometers mounted alone do not necessarily allow for the identification of intraspecific contexts in the interpretation of behaviour, falling short of what is often the hallmark of investigations of behaviour with regards to sociality (Pangle and Holekamp 2010; Fehlmann et al. 2017; Robinson et al. 2017). One solution may be to outfit several individuals within a group with multichannel sensors, as recently used in studies of flocking and schooling behaviour (Fujioka et al. 2016; Cook et al. 2017; Zaitouny et al. 2017). These studies using

multichannel sensors, however, assume that individual associations are stable over the study period. In the cases of animals with very loose associations and little sociality, the development of the so-called 'chat tags' have presented a useful co-opting of increasing data transfer in extreme environments to also investigate spatio-temporal behaviour and associations (Hazen et al. 2012). As with traditional behavioural observation methods, the research question must be rigorously considered to assess trade-offs in sampling frequency and data acquisition (Altmann 1974). The field of biologging is, however, a rapidly progressing field that may tackle many of these issues in the coming decade.

Data storage, battery life, and computer processing power represent some of the biggest limitations and trade-offs associated with the of accelerometers and other biologging channels and must be carefully balanced in order to meet the needs of the study (Sakamoto et al. 2009). A variety of analytical tools have been presented to assess these acceleration signals ranging from the impossibly time-consuming visual examination approach to more advanced machine learning techniques (Sakamoto et al. 2009; Graf et al. 2015). Machine learning techniques take two approaches: Supervised and unsupervised. Supervised machine learning can range from so called 'lazy learning' approaches, where training and classification occur simultaneously during run time and only classifies based on user-generated data using simple algorithms, such as with *k*-nearest neighbour algorithms (Bidder et al. 2014), to more black-box approaches where decisions are made independent of the user, such as random forest models or artificial neural networks (Cutler et al. 2007; Nathan et al. 2012; Wang et al. 2015; Brewster et al. 2018). Consistent among the supervised machine learning techniques, however, is the need for data to teach a program which signals are associated with specific behaviours through the use of a training data set. Unsupervised machine learning, however, does not require this training data in order to split the data into distinct classifications. Unsupervised approaches may still require statistical parameterization prior to implementation on a given dataset, such as support

vector machines or unsupervised cluster analysis using *k*-means methods (Sakamoto et al. 2009; Campbell et al. 2013), and return behavioural modes that are statistically different in the feature space of the data included. Interpreting these behaviours *post-hoc* is then up to the user.

Typically however, many studies of animal behaviour with tagging data have opted for supervised approaches. This is usually achieved by the deployment of an accelerometer on a captive individual who is trained and asked to perform the behaviours of interest so that an observer may then identify the specific acceleration signal to inform a consequent decision tree for analysis (Battaile et al. 2015). While the 'lazy learning' techniques are easier to implement with little programming knowledge, they seem less able to determine more complex behaviours or subtle differences in movement patterns. This may be due to the fact that these more simplistic classification algorithms rely on differences in classes, such as behavioural states, to be separated and clustered effectively in multidimensional space and do not handle data well that may overlap in certain dimensions. Event detection is also extremely difficult within the supervised machine learning approach. Individual behavioural events, such as an individual head lunge during prey acquisition, must be separately specified by either thresholding the larger dataset based on user-designed or optimized parameters or must be sampled at an extremely high rate in order to accurately detect that an event has taken place (Skinner et al. 2010; Viviant et al. 2010; DeRuiter et al. 2013; Ydesen et al. 2014; Shorter et al. 2017). As a result, many studies on animal behaviour have only been capable of resolving a few behavioural states or event types (Fahlman et al. 2008; Soltis et al. 2016; Fehlmann et al. 2017). In addition, individuals are rarely accessible to be directly sampled for behaviour during accelerometer deployments in the wild, which often leads to decision making that is lacking external validation. Some have proposed the use of surrogate species to aid in the development of training sets in difficult to observe or rare species that might not be easily

accessible in either a captive or wild situation, but the accuracy of using such species to build an accelerometry ethogram remains dubious (Campbell et al. 2013; Pagano et al. 2017). Several studies have attempted to classify behaviour of pinnipeds in captivity and extended this to wild, at-sea behaviour (Brewster et al. 2018; Ladds et al. 2018), but none to date have focused on the behaviour of pinnipeds during their terrestrial breeding phase of life history. This thesis aims to classify behaviour in a wild context by using this unique period of accessibility to evaluate behavioural trade-offs in this critical period of pinniped reproduction.

1.5 – The grey seal

Grey seals (*Halichoerus grypus*) are large bodied, sexually dimorphic members of the true seal family, Phocidae, and the only members of their genus. Distributed across the North Atlantic, the grey seal population can be divided into east and western distinct population segments breeding seasonally on either land or land-fast ice habitats (Hall 2002). Grey seals across the UK typically pup in the autumn, nursing their pup over an average 18 day lactation period in which females may lose 40% or more of their body mass (Mellish et al. 1999a; Pomeroy et al. 2000b). During this lactation period, grey seals exhibit what is known as a capital breeding system, where the energetic stores (fat or blubber and muscle) that they haul out on land with must sustain them for the duration of parturition, lactation, and mating (**Figure 1.1**); they do not return to the sea to forage for supplemental energy income through the duration of lactation (Bowen et al. 2006; Sparling et al. 2006; Stephens et al. 2009). Males compete for access to females, but do not necessarily defend geographically-discrete territories (Anderson and Fedak 1985; Twiss et al. 1994; Bishop et al. 2015b). Copulation typically takes place at the end of the lactation period. As a result, male-female interaction peaks towards the end of lactation for individual females and peaks towards the end of the breeding season for whole colonies (Bishop et al. 2015b, 2017). Following weaning, grey seal pups typically undergo a fasting period lasting between 10 and 28 days, sticking close to or on their natal site

(Hall 2002; Bennett et al. 2007). Grey seals also exhibit a high degree of fidelity, returning to their natal site to breed after reaching sexual maturity at the age of 5, though delayed recruitment is often observed (Pomeroy et al. 1994, 2010). Females will often return to the same location on the colony from year to year, but there is growing evidence to suggest that females choose sites within the colony that are close to sources of fresh water in order to drink and thermoregulate (Twiss et al. 2002; Stewart et al. 2014). Following copulation in the autumn, grey seals undergo embryonic diapause for 3 months and do not implant until after the completion of the annual moult and associated second fasting period (**Figure 1.1**). It is at this time that females are likely to have the least reserves and may abort the embryo and forgo pupping entirely for the next season if a minimum energetic threshold has been depleted (Boyd 1991, 1998; Pomeroy et al. 1999; Hall 2002). It is likely that females must balance energetic losses from one year with the energetic requirements to pup for the next year (Pomeroy et al. 1999, 2001; Desprez et al. 2018).

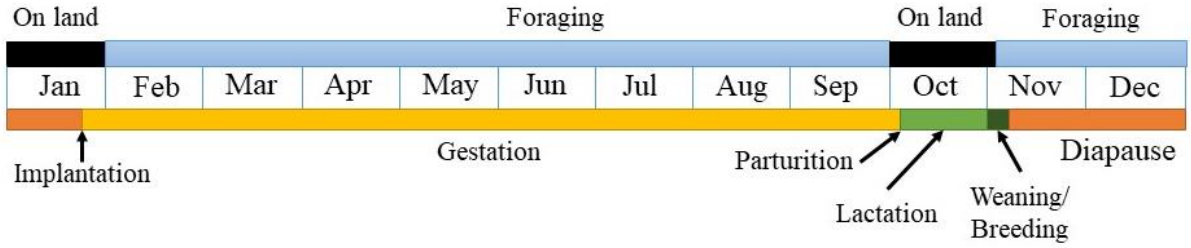


Figure 1.1: Annual cycle for an example adult female grey seal. Blue and black bars indicate the relative times of the year where individuals are foraging at sea or hauled out, respectively, for the moult in January and the breeding season in the autumn. Colour bars represent a typical breeding cycle. Females haul-out to give birth and nurse young in the autumn before weaning their pups, breeding, and returning to sea. During this stage, fertilized embryos remain in diapause until the end of the moult and subsequent implantation of the foetus before gestation begins in earnest.

1.6 – Assessing behavioural and energetic trade-offs

Long-term studies of wild individuals have been an important aspect of behavioural and evolutionary ecology in a variety of long-lived species (Amos et al. 1995; Twiss et al. 1998, 2007; Festa-Bianchet et al. 2000; Gaillard et al. 2000; Pelletier et al. 2009). Much of the focus in grey seal research has been how individuals optimize behaviour and energy expenditure to

maximize fitness in the context of sex or age class (Anderson et al. 1975; Anderson and Fedak 1985, 1987; Pomeroy et al. 1994; Twiss et al. 1994). Grey seals present a particularly good model system for long-term studies as they are colonial capital breeders that inhabit a variety of accessible coastal breeding sites across the North Atlantic making their energetic expenditures relatively easy to monitor (Boyd and Campbell 1971; Anderson et al. 1975; Boness and James 1979; Anderson and Fedak 1985; Pomeroy et al. 1994, 2000a; Twiss et al. 2012a). Because of these characteristics, they can be easily assessed at the start and end of lactation to track changes in body condition (Anderson and Fedak 1987; Lydersen and Kovacs 1999; Mellish et al. 1999a; Pomeroy et al. 1999; Schulz and Bowen 2004, 2005). Since they only have one pup per year, the energy expenditure for each mother and transfer efficiency to the pup can be directly quantified (Fogden 1971; Anderson and Harwood 1985; Kovacs 1987). Additionally, a high degree of site fidelity allows for long term, multiyear observations by uniquely identifiable pelage patterns (e.g., Anderson 1978; Pomeroy et al. 2000a; Beck et al. 2007; Twiss and Franklin 2010; Paterson et al. 2013). Capital breeding also presents a uniquely closed system in a wild animal in which to investigate energy allocation and usage and the trade-offs therein; female grey seals do not feed for the duration of lactation, therefore limiting all energy reserves to those acquired prior to the start of breeding. Any changes in physiological parameters as a result mean that individuals are directly trading off time and energy within a fixed energy reserve.

Work on this species can unfortunately be subject to the same limitations that other behavioural observation studies have seen. Observations typically only take place during the day, which during the autumn breeding season in high- and mid-latitude regions constitute roughly one third or less of a 24-hour cycle. Behavioural data can vary greatly across regions and study efforts, such that behaviour *in situ* can also be interpreted differently by a variety of observers, differing ethograms, study goals, and differing observation protocols limiting how

well these data can be collated between studies. Often studies examine short-term fitness during lactation by measuring mass loss over the course of lactation by sampling over two captures at the start and end of lactation, implicitly assuming a linear rate of mass loss and transfer (Anderson and Fedak 1987). While previous studies have noted that behaviour varies over the duration of lactation, it has remained very difficult to relate individual behavioural states to absolute energy used over a 24-hour period or over many consecutive days. Heart rate can be a reliable indicator of energy usage over time and can be tracked continuously animal-borne devices (Portugal et al. 2016; Twiss et al. *in review*). The use of accelerometers in conjunction with heart rate monitors in the grey seal system seeks to overcome these challenges and provide fine-scale and high-resolution pictures of behavioural and energetic patterns over lactation.

The potential to gather unbiased behavioural data across the entire day throughout lactation could also allow for continuous estimation of how energetic expenditure might change across the breeding season and may reveal potentially critical periods and differing maternal investment strategies within a population. Very little is known about fine-scale temporal trade-offs in behaviour or energy usage over lactation together as a single holistic picture. While other studies have shown that mass transfer and milk energy density changes across the lactation period (Mellish et al. 1999a; Bennett et al. 2007), it is unclear how much of an effect individual behaviour may have in predicting individual differences in energy transfer from female to pup. There is a multitude of evidence to suggest that energy expenditure is not uniform over lactation (e.g. Iverson et al. 1993; Mellish et al. 1999b), but it still remains unclear if any such changes are purely driven by local environmental stimuli, such as temperature or water access (e.g. Stewart et al. 2014), or if there is any evidence that individually different strategies of investment exist within the larger population of grey seals. Differences in patterns of maternal mass loss has already been suggested to correlate along the

range of proactive and reactive behavioural types in breeding female grey seals (Twiss et al. 2012a). Data acquired from accelerometers will allow for the investigation of the potential life-history strategy trade-offs associated with fine-scale individual differences in behaviour and their consequences for the next generation (Levin et al. 2000; Dall et al. 2004; Twiss and Franklin 2010; Twiss et al. 2012b).

1.7 – Research aims

Overall, this thesis seeks to investigate fine-temporal scale behavioural and energetic trade-offs through the lens of tri-axial accelerometers and heart rate monitors in lactating grey seals over three consecutive breeding seasons. The protracted lactation period in Phocid seals makes this species an excellent proving ground for a variety of techniques, likely giving unprecedented insight into trade-offs previously unattainable through traditional observational methods. A variety of techniques have been used in this thesis to address the knowledge gaps previously discussed. Animal-borne accelerometers seek to overcome the inherent difficulty of monitoring behaviour continuously for a full 24-hour period, while heart rate monitors will give useful samplings of physiology without the need for repeated handling events. Assessing the fine-scale energetics and potential selection trade-offs associated with behavioural shifts may aid in a higher resolution understanding for management of northern latitude species like the grey seal where patterns and implications discussed here are likely extensible to other sensitive pinniped species (discussed extensively in **Chapter 7**). In total, this thesis can be broadly grouped into two main sections. Here, I will briefly outline the main objectives of the thesis and the techniques used to address and elucidate time and energy trade-offs in lactating grey seals.

Section 1: Behavioural trade-offs in time for a capital breeding species (Chapters 3, 4)

This first section aims to build tools to evaluate behavioural trade-offs over lactation. More specifically, I aim to develop unbiased methods using animal-borne accelerometers for

assessing behaviour at an equivalent temporal resolution to that typically afforded with traditional behavioural observation methods (**Chapter 3**). Behavioural classification of accelerometry data will be achieved through advanced machine learning techniques and validated through the using direct observation via video footage gathered in the field. Using the fine temporal-resolution behavioural data from accelerometers, I wish to assess trade-offs in activity budgets across the duration of lactation (**Chapter 4**). By examining high-resolution activity budgets over time through the lens of possible intrinsic drivers of behaviour, such as the sex of the pup and the female's inherent stress-coping styles, as well as extrinsic drivers of behaviour, like temperature and colony location, it is my hope to identify and quantify discrete trade-offs in time allocation and their drivers.

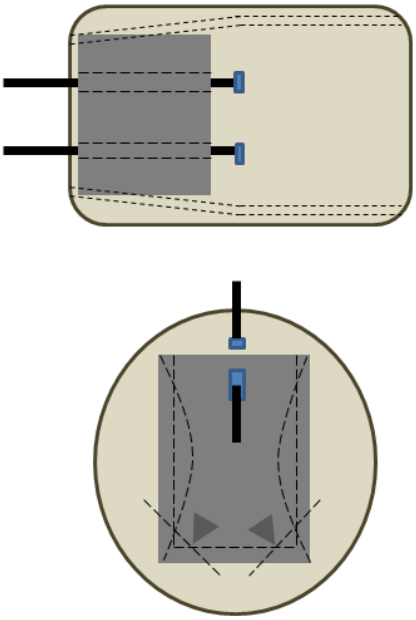
Section 2: Drivers and consequences of energy allocation during lactation (Chapters 5, 6)

The second section of this thesis aims to tease apart the physiological drivers of energy budget of females over lactation using animal-borne accelerometers and heart rate monitors without the need for repeated sampling events. First within **Chapter 5**, I wish to characterize energy usage over lactation by tracking changes and consistency in activity levels over time between years. Following this, I will use a combination of heart rate data and accelerometry to build a holistic picture of whole animal energy management strategies within a capital breeding system as the main mechanism for assessing trade-offs in energy allocation and usage within changing activity levels. Using these models of energy allocation, I wish to further investigate how these energy resource allocations respond to temperature across the colony and how individuals with differing stress-coping styles vary in energy usage. Finally, this assessment will be extended to how energy management may have an effect on short-term fitness outcomes associated with maternal mass loss rates and mass-transfer efficiency to the pup. While changes in energy usage have been assumed to be a straight line between early and late lactation, I hypothesize that critical periods likely exist in which energetic expenditure is higher

than expected by the assumed linear relationship. I also expect that different stress-coping styles, already known to display differences in behaviour will also lead to vastly different strategies in managing and trading off energy usage over lactation. To further delve into trade-offs in energy allocation, **Chapter 6** aims to assess whether accelerometers can be used alone in order to detect changes in heart rate as well as detecting individual breaths while at rest. Changes in heart rate are closely tied to breathing rate and both remain a rarely accessible physiological trait in a free-ranging species. As grey seals regularly alternate between periods of breath hold and breathing while on land, I also evaluated the function apnea at rest (periods of breath-hold) in relation to energy allocation and environment. This was done in order to investigate the potential energy and water conservation benefits of retaining this dive reflex on land and may further illuminate individual mechanisms for energy management over this contracted and extremely high energy output period of life history.

Chapter 2:

General Methods



2.1 – Study colony

This study focused on lactating adult female grey seals on the Isle of May in Scotland (56.1° N, 2.55° W), located on the outer Firth of Forth and managed by Scottish Natural Heritage as a National Nature Reserve. The island is characterized by highly variable, mixed terrain with broken rocky areas and open tussock along its Northern and Western shore sloping up to vertical cliffs to the Eastern and Southern ends of the island. During the breeding season, the majority of seals are found to aggregate along the approximately 6 ha area known as Rona at the North end of the island, while others can be found on the Southeastern end of the island in several regions subdivided by various physical landmarks and man-made barriers (Pomeroy et al. 2000a; **Figure 2.1**). Access to fresh water is variable across the island with some aggregations of seals close to inter-annually consistent large pools, while others are only found near ephemeral puddles due to a wide range of topographical variation across the colony. Pup production steadily increased on the island since the colony was first studied in the late 1980s, becoming one of the larger colonies in the UK with approximately 2,000 pups produced per year (Kovacs 1987; Pomeroy et al. 2000a). More recent projections of survival and low colony recruitment appear to indicate that the population is no longer growing (Pomeroy et al. 2010). While open to tourism as a popular viewing area for the large seabird colonies that take up residence during the summer months, the Isle is closed to tourism during autumn seal breeding season to minimize disturbance. Human disturbance during the breeding season is therefore minimal compared to other more easily-accessible, coastal colonies such as Donna Nook in Lincolnshire (Bishop et al. 2015b). Adult grey seals typically begin to arrive on the island in early October to pup and mate, with peak density around mid-November, and slowly declining until mid-December (Pomeroy et al. 2000a). This study focused only on lactating females within the core period of breeding between late October to early December for three consecutive years between 2015 and 2017. Lactating females were chosen at various sites

within the island colony with varying topographical features, water accessibility, and presence of man-made barriers (see **Figure 2.2** for specific capture locations within the Isle of May across years).

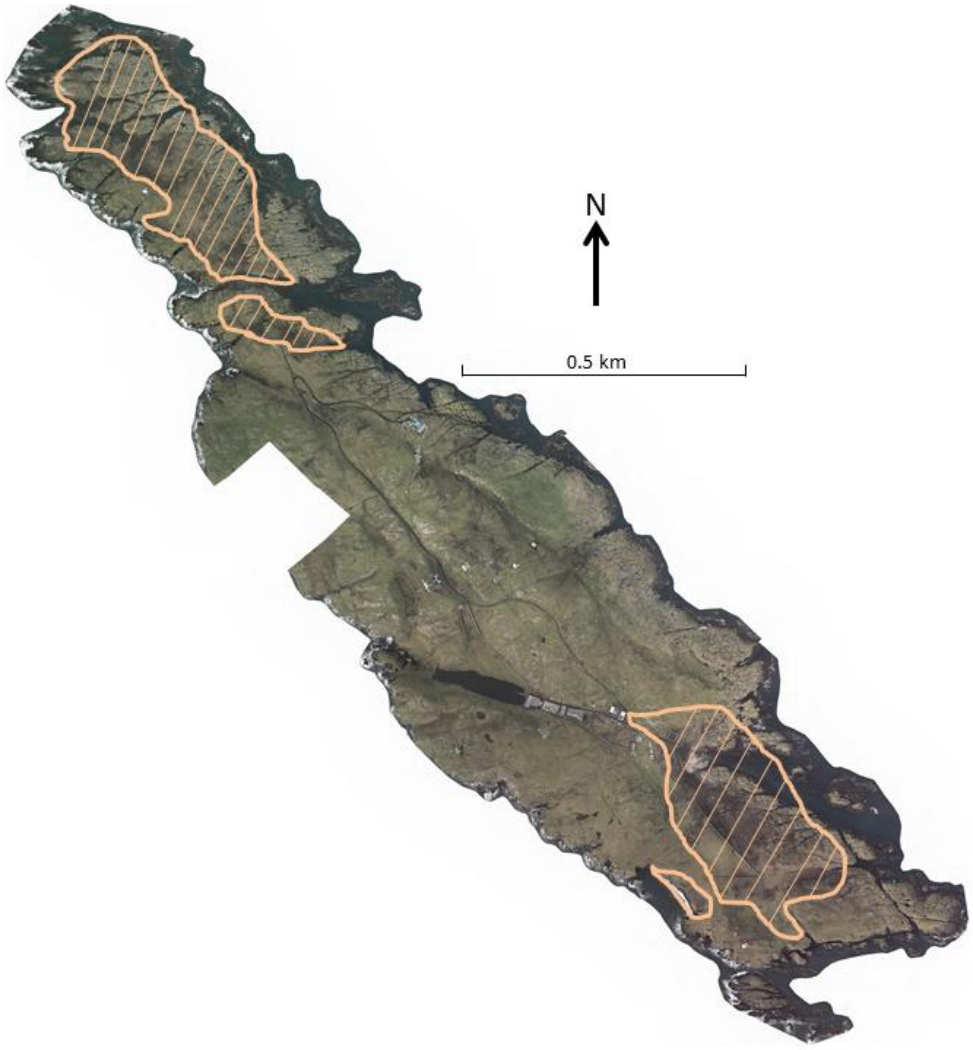


Figure 2.1: Full map of the Isle of May (IoM), Scotland (56.1° N, 2.55° W) adapted from Pomeroy et al. (2000). Main areas of grey seal populations are roughly outlined in orange. The white square cutout towards the center of the island was not sampled in satellite composite and therefore represents missing data. Further detail indicated in **Figure 2.2** with man-made barriers and individual capture locations for each year of study (Photo: SMRU aerial survey composite).

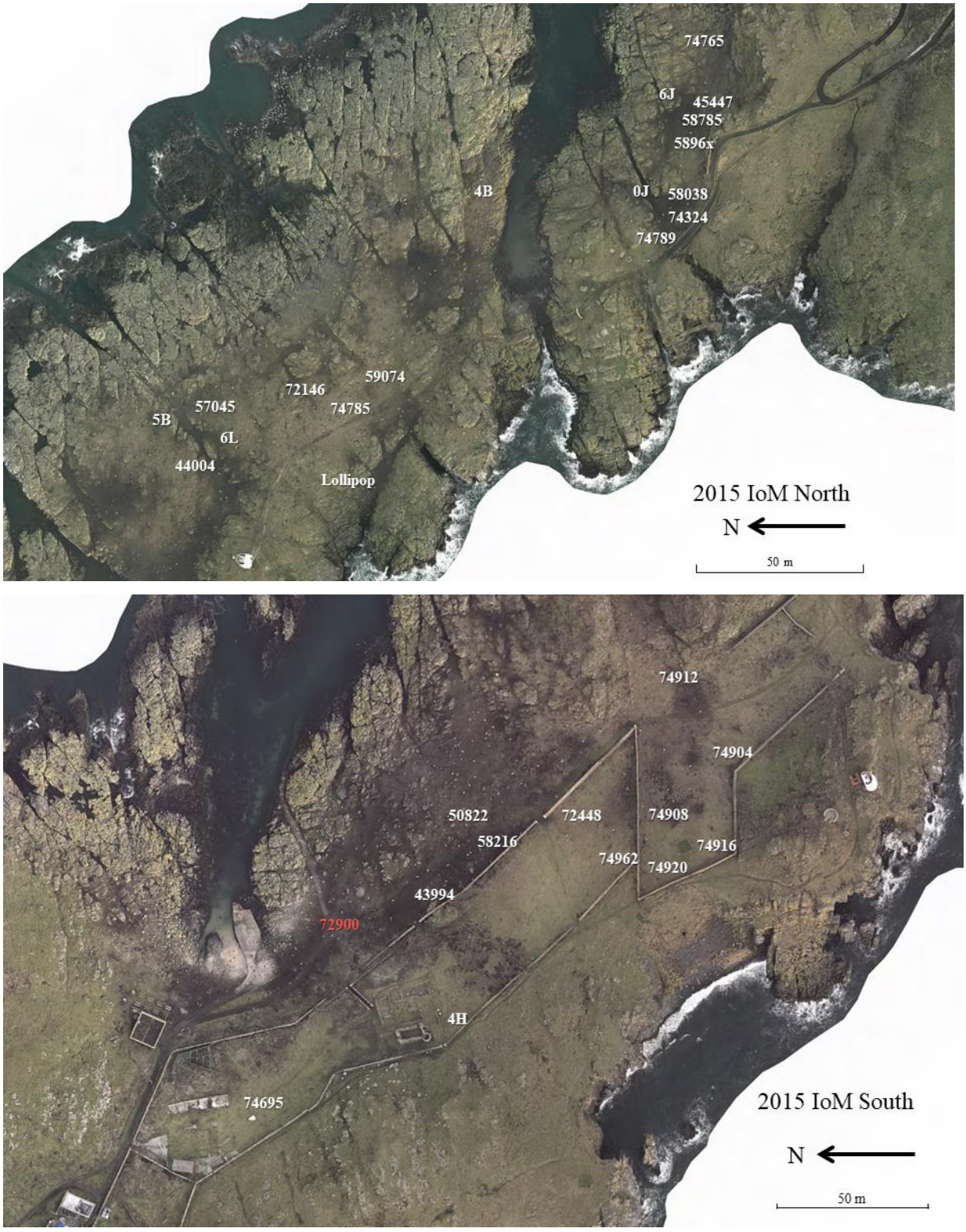


Figure 2.2a: Map of study females in the north (top) and south (bottom) of IoM for 2015. Seal IDs in red indicate that the female departed prior to recapture and no accelerometry data was available (Photo: SMRU aerial survey composite).

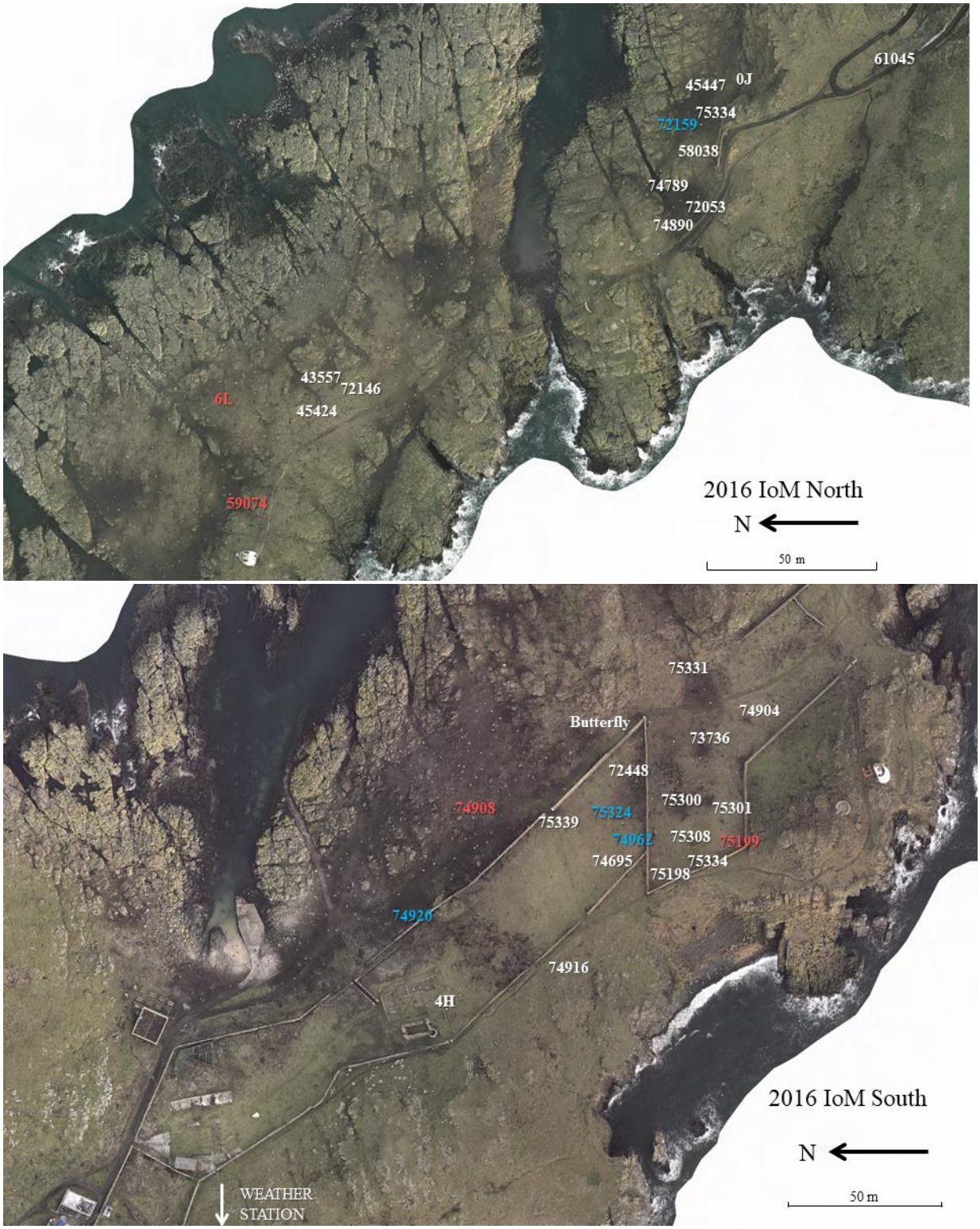


Figure 2.2b: Map of study females in the north (top) and south (bottom) of IoM for 2016. Seal IDs in red indicate that the female departed prior to recapture (no accelerometry data), while those in blue were recaptured early (partial). The location of the weather station is also indicated (Photo: SMRU aerial survey composite).

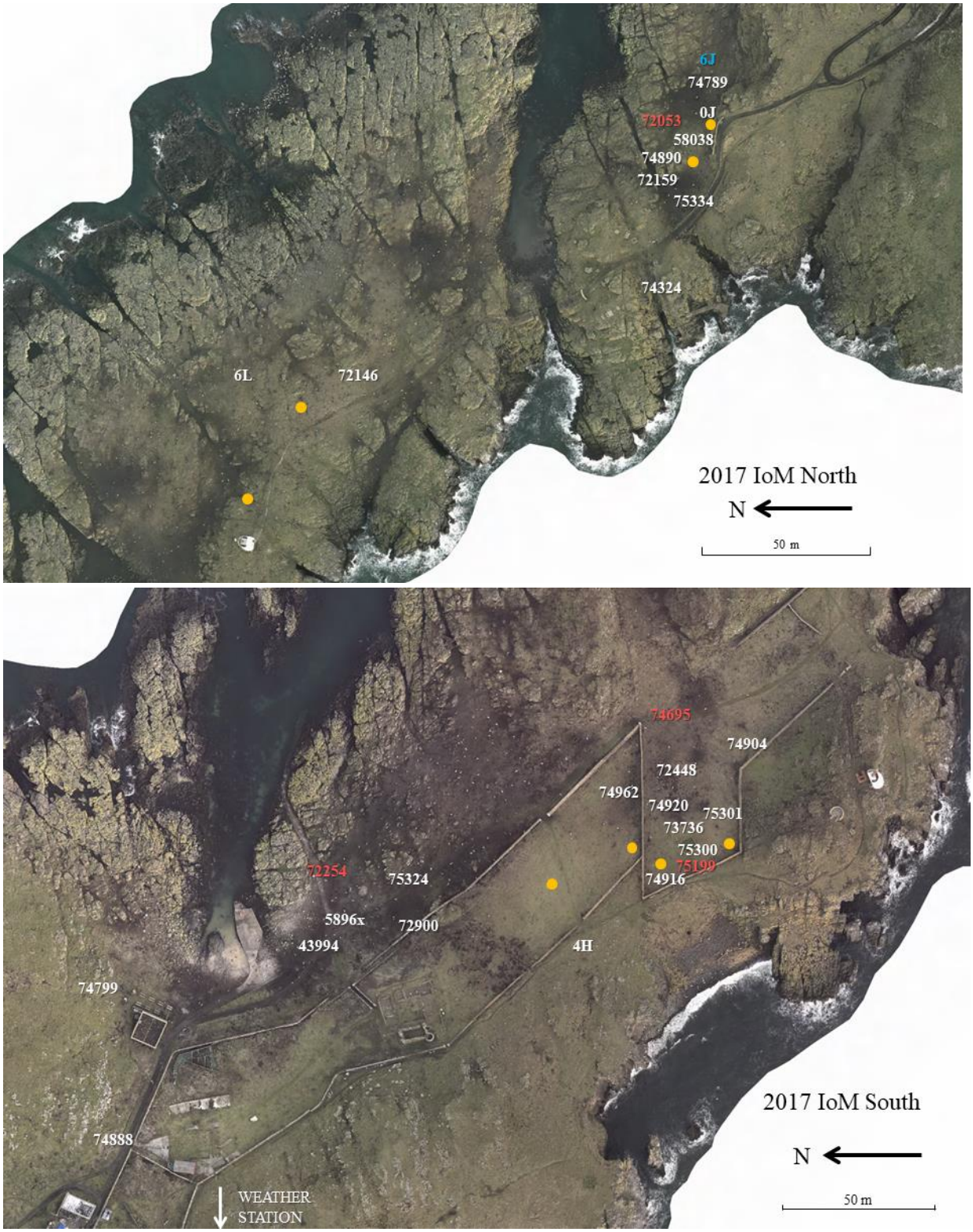


Figure 2.2c: Map of study females in the north (top) and south (bottom) of IoM for 2017. Seal IDs in red indicate that females departed prior to recapture (no accelerometer data), while those in blue were recaptured early (partial). Orange dots indicate iButton temperature logger locations in the colony as well as the weather station (Photo: SMRU aerial survey composite).

2.2 – Study animals

Adult females were sampled both early and late in lactation to capture the core of each individual's approximate 18-day lactation period (Pomeroy et al. 2000a; Bennett et al. 2007). Each animal was captured via chemical immobilization using a blow-pipe and intramuscular injection dart containing mass-specific dose of zolazepam-tiletamine ('Zoletil', 1.0 mg ml^{-1}, Virbac, U.K.; Pomeroy et al. 2000a). Upon sedation, animals were then weighed using a portable digital hanging scale (± 0.2 kg, Straightpoint Miniweigher Plus, MWP500, 0-500 kg) and tripod. Each pup was also weighed, sexed, and classed into a developmental stage at each capture event (I-IV; Boyd and Campbell 1971; Kovacs and Lavigne 1986; Woldstad and Jenssen 1999) to assess mass transfer efficiency over the lactation period (ratio of maternal mass loss to pup mass gains). Blood serum samples, photogrammetry, morphometrics, and blubber depth via ultrasound (for 2015 season only) were also gathered in each sampling occurrence to assess body condition and composition as part of a larger study, however elements of body condition were not included in this thesis. Further information on capture methodology can be found in Bennett et al. (2007). Accelerometer and heart rate monitor attachment took place at the initial sampling event. All telemetry devices were then removed at the final handling event late in lactation. Females were given an identity (ID) either based on a unique alphanumeric brand found on the flank of the individual (e.g. '6L'), the five digit serial number located on the left interdigital tag located on the rear flippers (e.g. '74789'), or by a unique name allocated by the larger collection of uniquely identifiable pelage or scar patterns (e.g. 'Lollipop'; see **Figure 2.2**). As tag loss was experienced in this study between years, unique IDs were assigned based on the above characteristics from first capture in this study. All recaptures were further confirmed by comparing unique pelage patterns between years.

All applicable international, national, and/or institutional guidelines for the care and use of animals were adhered to in this study. All animal procedures were performed under UK Home Office project license #60/4009 and conformed to the UK Animals (Scientific Procedures) Act, 1986. All research was approved ethically by the Durham University Animal Welfare Ethical Review Board as well as by the University of St. Andrews Animal Welfare and Ethics Committee. Only highly trained and experienced personnel performed all capture and handling procedures of the grey seal mothers.

2.3 – Tag deployments

Two separate models of accelerometers were used in this study, depending on the year, including the AXY-Depth model accelerometer (32 x 14 x 13 mm, mass of 7 g each; Technosmart Europe, Italy) or GPS-enabled accelerometer AXY-Trek (63 x 37 x 13 mm, mass of 50 g each; Technosmart Europe, Italy). Accelerometer data loggers measure both dynamic acceleration (movement) and static acceleration (posture and position) in three axes: heave (dorso-ventral movement), sway (lateral movement), and surge (anterior-posterior movement). All accelerometer configuration was undertaken through Technosmart's software package 'AxyManager2' (https://www.technosmart.eu/). Prior to deployment, each pre-calibrated accelerometer (calibrated by Technosmart) was configured to sample acceleration (g) at either 25 or 50 Hz in all three dimensions on a continuous regime for the length of the deployment with a g range of either ± 2 g or ± 4 g depending on the year. In addition to factory calibration, each tag was tested for excessive floor noise by being placed on a level surface for 30 minutes undisturbed as well as undertaking real-time calibration flips in all dimensions; none of the tags appeared to behave abnormally and no further calibration of the data was deemed necessary. Sampling rate was originally set to 50 Hz as this appeared to achieve sufficient detail in accelerometry features from previous work (e.g. Skinner et al. 2010) and available settings, while still covering the majority of the planned 10 day deployment of tags. However, a glitch

in the firmware, as discussed later in this thesis, resulted in several tags turning off during the 2015 season as a function of sampling at this high rate. The tags still functioned at lower sampling rates, but this issue was not resolved until after the 2016 season resulting in individuals being sampled at half the rate (25 Hz). Since all animals were mostly restricted to land during their lactation period, pressure and temperature sensors were turned off in an effort to maximize battery life and storage capacity. After configuration, the serial port on each accelerometer was packed with silicone grease and covered with a thin layer of epoxy to prevent grit from contaminating the pin plugs. Tags were housed in custom-designed ballistic nylon footprint housing pouches and attached onto dry pelage using superglue (Loctite, formula 422; **Figure 2.3**). Protocols differed from year to year for the number and placement of accelerometers (see **Table 2.1** for sample sizes and **Figure 2.4** for attachment examples). In 2015, each accelerometer was placed on the top of the head on the midline to align the positive x-axis (surge) of the tag to the forward motion of the individual (henceforth referred to as 'head-mounted'; **Figure 2.4a**). In 2016, the majority of individuals received only head-mounted accelerometers. Ten females, however, were equipped with one additional accelerometer mounted forward of the shoulders in the same alignment (henceforth referred to as 'torso-mounted'; **Figure 2.4b**). Prior to completion of these double deployments, accelerometers were tapped in unison to allow for *post hoc* synchronization of acceleration signals for comparison purposes. In 2017, all but four individuals received a single torso-mounted accelerometer (**Figure 2.4c**). Two of these individuals received one GPS-enabled accelerometer (AXY-Trek, TechnoSmart Europe, Italy), aligned as above, in tandem with one additional accelerometer as a trial of its accuracy placed in the same pocket with the positive y-axis (sway) aligned with forward movement. The other two only had a single GPS-enabled accelerometer mounted in front of the shoulders with the x-axis aligned with forward

movement (**Figure 2.4d**). The time the unit was turned on was marked with a watch that was regularly set to an atomic clock to allow for synchronization to the video footage.

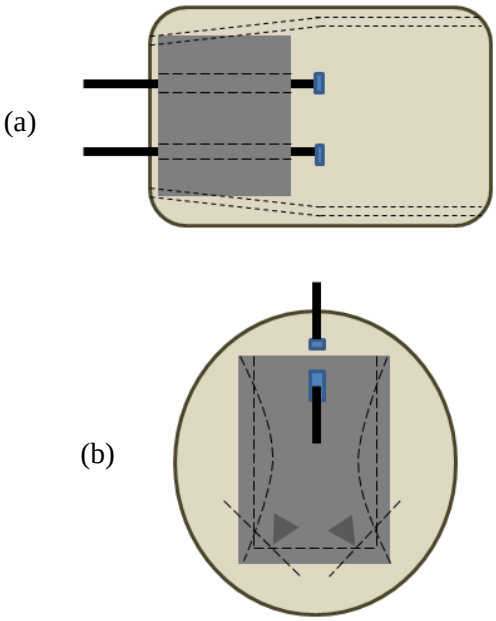


Figure 2.3: Schematic of custom designed ballistic nylon housing for the (a) heart rate monitor and (b) AXY-Depth model accelerometer.

Figure 2.4: Examples of deployments for (a) 2015 head-mounted accelerometer only deployments, (b) 2016 double accelerometer deployments with one head and one torso mounted accelerometer, (c) 2017 torso mounted accelerometers only, and (d) a larger GPS-enabled torso-mounted accelerometer. Heart rate monitors also pictured in all panels with leads extending down the sides of flanks (Photos: CR Shuert and SD Twiss).

Each heart rate monitor was placed on the back with the central transmitter housing on the midline with leads extending down both flanks with a total tag mass of about 100 g (modified Firstbeat Technologies; **Figure 2.4**). Electrodes were treated in a solution of 11% sodium hypochlorite for at least 3 hours in order to create a continuous surface of silver chloride prior to each deployment (Halsey et al. 2019; Twiss et al. *in review*). A small section of fur was clipped by hand to match the size of each electrode at the end of each lead, and placed just behind the armpits of each female on both sides of the body. Each electrode was held in place with a small plastic doughnut glued onto the fur, packed with medical-grade electrogel (Ten20, Weaver and Company, Aurora, Colorado), and secured with a cover plate and screws. These heart rate monitors give inter-beat-intervals (IBIs) based on the detection of R peaks, rather than a typical QRS-curve as found with an ECG signal. Post-processing allows for the removal of artefacts and errors in the detection of these IBIs; only those traces with less

than 50% flats (invariable sequences of IBIs) and stairs (monotonically increasing or decreasing IBIs) were included in all subsequent analyses using custom designed R scripts as part of a larger effort, as these were considered to be errors either due to movement or poor surface contact (see Twiss et al. *in review*; R Core Team 2016; Brannan 2017). Unlike the accelerometers, the heart rate monitors transmit the data to a portable receiver station (Firstbeat Team Receiver) and do not log any of their recordings. This receiver station can pick up tags that are up to 200 m away in line of sight. Due to the size and topography of the island, all animals could not be recorded simultaneously for the entire length of their deployment. Upon recapture prior to weaning her pup, the tags from each female were removed whereupon the accelerometry data could be downloaded and processed. Heart rate data were validated using a portable ECG during recapture events (AliveCor Mobile ECG and iPod Touch; Twiss et al. *in review*). A summary of each year's sampling effort is listed in **Table 2.1**. Maximum tag load across the study years was 160 g, well under 0.001% tag to body mass ratio for the smallest female in the study (Horning et al. *in press*; Portugal and White 2018).

Table 2.1: Summary of accelerometer sampling effort over the three-year study period on the Isle of May. Each year lists the total number of females with either one or two accelerometers deployed. 2015 only used head-mounted accelerometers, while 2017 only used torso-mounted accelerometers. Double deployments (2x ACC) in 2017 included the use of 1 GPS-enabled accelerometer in conjunction with a separate tri-axial accelerometer. The number of females that were recaptures from the previous study year is included in brackets. Individuals were counted as losses if they left the study site prior to the anticipated recapture date. No accelerometer data could be obtained if the tags were not retrieved (losses).

	Study Year			Total Deployed
	2015	2016	2017	
1x ACC	29	14 (3)	22 (19)	*65*
2x ACC	0	10 (8)	2 (2)	*12*
Losses	1	4 (3)	4 (3)	*9*
Total Females Sampled	30	28 (14)	28 (24)	***86***

2.4 – Focal video sampling *in situ*

Over the deployment period for all three years, each individual was sampled for behaviour using focal sampling approach for at least 3 dedicated sessions during the daylight hours (Altmann 1974). Videos were recorded using a digital high definition video recorder (Panasonic HC-V700, 1920x1080 resolution with 46x zoom, 28 mm wide lens; Panasonic Corporation) on a tripod from at least 50 m away from the focal individual and archived to be decoded for time-activity budgets. Since multiple individuals were tagged simultaneously, every effort was made to visit each individual as frequently as possible over the tag deployment period. At the same time, the heart rate monitor receiver set up was also in use to record real-time heart rate on individuals within a 200 m radius during the video recording period (see **2.3**). Contextual notes were also made to aid in decoding the videos for behaviour after returning from the field.

2.5 – Decoding behaviour

2.5.1 – Behavioural ethogram

One of the main goals of this project was to compare the efficacy, precision and accuracy of behavioural categories gleaned from accelerometry to more traditional behavioural observation techniques. As a result, focal video footage was sampled for behaviour at a fine-scale of behavioural resolution including a wide variety of contextual and social behaviours as part of a larger effort to document behaviour in lactating grey seals. The behaviours were defined *a priori* based on previous work by Kovacs (1987b) and Tinker et al. (1995). Behaviours were sampled to the nearest second.

A) **Non-Aggressive Behaviours**:

A.1 Resting:

*Rest (**REST**)* – Seal is motionless, head down on ground, eyes closed. Can be situated in any rotational axis (e.g. on back or side), however with no pup interaction (e.g. not presenting to pup for nursing – *N/P*). Similarly defined as 'idle', but eyes remain closed (Tinker et al. 1995).

A.2 Alert Behaviours: *In an effort to potentially separate mechanistic differences in posture, 'Alert' as defined in previous studies* (Anderson and Harwood 1985; Kovacs 1987) *was split into separate sub-categories.*

*Alert Head Up (**ALHU**)* – Seal is stationary, not changing physical orientation or geographic position, with head up and eyes open. This is usually a transition from either a rest or an *ALHD* behaviour.

*Alert Head Scanning (**ALHS**)* – Seal is stationary in geographic position and orientation, but head is up, actively moving, panning and scanning head around beyond small movements, observing the surroundings, looking at conspecifics, disturbance sources, etc. Seal may execute a pup check during or at the end of the scan (which should be marked as *PC*).

*Alert Head Down (**ALHD**)* – Head down, but eyes open. Small movements of the head are included, but if movement is frequent and extends beyond minor movement, this should be classified as *ALHS*.

*Pup-Check (**PC**)* – Directed head movement where eyes are open and the seal moves its head and direction of gaze toward location of pup. This can be quick (~1-2 sec) or longer, but each look considered to be a separate pup check. This behaviour can only

be differentiated from other alerts if the pup location is known. If out of frame, pup location was noted in field sampling notes. This is used as an important metric for individually consistent behavioural types as used in Twiss et al. (2012).

A.3 Movement:

*Locomotion: (**LOCO**)* – Seal changes geographic location (>0.5 body lengths) without obvious directed conspecific interaction upon commencing of behaviour. Seal is moving on its ventral surface using its fore- and hind-flippers or shuffling along on its lateral surface with one flipper tucked up underneath and no flippers in use to aid in movement. This movement is generally in a single direction. If directed at other individual(s) or after confrontation with individual(s), classify as *Chase* or *Flee*, respectively.

*Roll: (**ROLL**)* – Rotation of body around the long axis of the body where the seal changes geographic location.

*Comfort Movement: (**CM**)* – Female in a resting or alert position performing low intensity movements that do not change its geographical location (<0.5 body lengths) and includes scratching, stretching, rolling, etc. as previously defined (Tinker et al. 1995). This also includes changing orientation where an individual may rotate more than 45 degrees from original orientation at the onset of the behaviour.

A.4 Mother-pup Behaviours:

*Nose Pup (**NOP**)* – Directed head movement toward direction of pup where nose comes into contact with her pup. Behaviour can include head movement in line with pup to maintain contact.

*Flippering Pup: Flip-P (**FP**)* – Female using fore-flipper to lightly stroke/scratch pup. Often precedes nursing event but may also be used to encourage movement while female is attempting either non-aggressive locomotion or in an aggressive interaction context.

*Mother-pup Interaction: MPint (**MP**)* – Any other active interaction between female and her pup where female is in direct physical contact not defined by other specific behaviours. This can include play-like behaviours like light biting, weak open-mouth threats to pup, climbing on pup, placing a flipper on pup that are executed in combination. There is no separate category for play.

*Presenting/Nursing (**PN**)* – Female rolls laterally so that nipples are presented to the pup, usually with the female resting on one flank and is designated as such when the female is within one pup body length at an approximate perpendicular orientation to the pup. This behaviour can also occur with several Comfort Movements (*CM*) and Pup Checks (*PC*) to position correctly for nursing and should be specified within this behavioural state. *Nursing (**N**)* – Female already in presenting position described with nipples exposed, pup is in direct oral contact with mother and appears to be suckling or moving between nipples using nose to push on mother between attachment. Female often appears to be resting during nursing bouts. There has been no attempt to quantify the amount of milk that was transferred to the pup from the female. This behaviour only indicates the time latched on to the nipple. For the purposes of this ethogram, presenting and nursing are pooled into the same behavioural state despite being grouped into two separate behaviours in other investigations (Kovacs 1987).

*Presenting/Nursing Alert: (**PNAL**)* – Same as Presenting/Nursing *(PN)*, but head is raised from a horizontal position with eyes open and is considered Alert (*ALHU/ALHS*) scanning the environment.

A.5 Other Non-aggressive Behaviours:

*Nose Female (**NOF**)* – Non-aggressive directed head movement and sniffing toward conspecific adult female where noses or whiskers come into contact to those that sniffing is directed to.

*Nose Male (**NOM**)* – Non-aggressive directed head movement and sniffing toward conspecific adult male where noses or whiskers come into contact to those that sniffing is directed to.

*Nose Other (**NOO**)* – Non-aggressive directed head movement and sniffing toward any other conspecific including non-related pup, recently weaned pup, or sub-adult where noses or whiskers come into contact to those that sniffing is directed to.

B) **Aggressive Behaviours**: The first four behaviours in this category include designations to indicate the type of conspecific a behaviour type is directed toward. This includes direction of behaviour toward adult males, adult females, or other pups (e.g., *OMTfemale*, *BImale*).

B.1 Threat Behaviours

*Open-mouth Threat (**OMT**)* – Female will open mouth, sometimes baring teeth to a potential threat, often accompanied with a vocalization directed at another individual.

*Aggressive Flippering (**FA**)* – Female uses either fore-flipper to rapidly wave at a potential threat. Flipper can either make contact with threat or can flipper the air (no contact), can also include *OMT* during this behaviour.

*Slapping (**SL**)* – Female either lying laterally on her flank or on her back and uses either fore-flipper to repeatedly and quickly beat her flank, producing a loud slapping or cracking noise. Slapping often accompanied with vocalization coupled to Open-mouth Threats (*OMT*).

*Flippering Ground (**FG**)* – Female uses either fore-flipper to scratch at the ground quickly and often repeatedly. This often results in tearing up the substratum coupled with Open-mouth Threats (*OMT*), typically observed during the approach of a threat or con-specific.

B.2 Fight Behaviours

*Bite/Lunge (**BI**)* – Adult female will rapidly extend the neck towards a potential threat with an open gape. If no physical contact is made, this is considered to be a Lunge (*Lu*). If female makes contact with teeth to the potential threat and closes jaw, this is considered to be a Bite (*Bi*). The two are lumped due to the similarity in mechanics despite different outcomes.

*Fight (**FI**)* – Female makes prolonged aggressive physical contact with another adult conspecific. Can include a rapid series of Bite/Lunge (*Bi/Lu*), Aggressive flippering (*FA*), and Open-mouth Threats (*OMT*) in succession and in various combinations with little to no time between successive behaviours. These are lumped into one due to their rapid and potentially undiscernible nature during extended aggressive physical contact.

*Chase (**CH**)* – Female pursues a potential threat by directed rapid locomotion towards threat. Threat source can either remain still, holding ground against chasing female, or be pursued for extended rapid locomotion by pursuing focal female. This behaviour was previously defined in Tinker et al. (1995), but without distinguishing the gender of the threat source.

*Flee (**FL**)* – Focal female is target of a Chase (*CH*) behaviour from another individual, resulting in rapid directed movement away from threat source. May look back as pursuit takes place. Previously defined as such in Tinker et al. (1995), but without differentiation into female and male pursuit.

C) **Sexual Behaviours**:

*Attempted Copulation (**ATCop**)* – Typically male initiated, though female may be cooperative or may actively approach male. Male is attempting to mount female which may or may not end in a successful Copulation (*Cop*). This is often accompanied by other aggressive behaviours while male attempts to gain proper position alongside the female. Behaviour also defined as 'mount female' in Tinker et al. (1995).

*Copulation (**Cop**)* – Typically observed at the point where male and female coupling becomes relatively stationary for an extended period while in a copulatory embrace (male holding female alongside with less movement than in Attempted Copulation (ATCop)), where male achieves intromission. This behavioural category does not attempt to categorize whether or not successful ejaculation occurred, nor a 'successful' breeding event, as defined in Tinker et al. (1995).

D) **Other Behaviours**:

Other – Any other behaviour not included in the preceding. This can also include new or previously unobserved behaviours. Some *Other* behaviours may include drinking from a freshwater source or scratching the substrate with fore flipper while nosing the ground. Where possible, notes of these 'new' behaviours have been made with reference to time and video location where behaviour occurred for later review and revisiting.

*Out-of-sight (**OOS**)* – Female is no longer in the video frame or is hidden behind an object or terrain feature or other individuals. This can include where the entirety of the animal is not visible as well as cases where part (e.g. head) is not visible or obstructed in order to accurately determine behavioural state.

2.5.2 – Video-derived time-activity budgets

Using the behaviours defined in **2.5.1**, videos were watched in real time for each focal individual and behaviours recorded using a custom-designed Visual Basic program to record the change in behavioural state in real time with the videos to the nearest second. Videos were re-sampled when more than one tagged animal was in frame as a separate focal video sample. Notes taken in the field were used to interpret context outside of the frame when needed. Time-activity budgets were calculated by pooling the total amount of time spent in each behavioural state from each focal video as a proportion of the total observation period (Bishop et al. 2015b; Culloch et al. 2016; Robinson et al. 2016), excluding the time out of view. Since little work has been done to resolve differences in day and night behaviour, the focal sampling only represents the daytime activity budgets of the adult females (Anderson 1978; Culloch et al. 2016). A total of 10% of the video footage was re-watched between 2015 and 2016 to check for consistency in behaviour decoding, resulting in an average difference in cumulative

time spent in each behaviour of about 5 seconds per video (approximately 0.07 ± 1.8% difference in activity budget), with moderate agreement (Cohen's kappa = 0.57). Behaviours were initially sampled using all the above context-specific behaviours. Further ethograms were generated by collapsing behaviours into coarser groupings of the categories in the full ethogram in order to assess a range of scales of behavioural detection finesse that could be achieved by the accelerometers (see section **2.5.3**).

2.5.3 – Behavioural classification scheme

In order to assess the levels of resolution of behavioural detection sensitivity with the accelerometers, a behavioural classification scheme (BCS) was designed *a priori* for comparison between the video-derived time-activity budgets and those derived from the accelerometers. These were based on *a priori* considerations on the mechanics of movement that were hypothesized to be more important in the accelerometer context for differentiating behaviours, rather than specific social contexts. These larger categories are outlined in **Table 2.2** moving from context-specific behaviours to more coarse categories. The first level was a simple active and inactive state classifier in which all other behaviours outside of what is defined as rest (Behaviour **A.1–REST**) were classified as an active state (BCS01, **Table 2.2**). Three other behavioural classification schemes were developed based on *a priori* decisions on potential resolution of behaviour within the scope of detectable movement and mechanics. These three included a coarse- (BCScoa), medium- (BCSmed**)**, and fine-level (BCSfine) of classification in which the total number of possible behavioural states was limited to 8, 10, and 15, respectively (**Table 2.2**). The final classification scheme representing the global model of behavioural detection sensitivity included all 27 context-specific behavioural states defined in the full ethogram (BCScon; **Table 2.2**), however no distinctions for interactions with different conspecific categories were included (males, females, or pups). While behaviour was sampled in video footage as part of a larger effort to document fine-resolution behaviour of female grey

seals, some behaviours were removed from further analysis due to their extremely rare occurrence in a females activity budget (< 0.0001% of total activity budget) including slapping (Behaviours **B.1–SL**), attempted copulation and copulation (Behaviours **C–ATCop** and **COP**; see **Table 2.2** for activity budgets between years). This meant that each of the behavioural classification schemes were reduced to 2, 7, 9, 13, and 25 behavioural categories, respectively. These 5 behavioural classification schemes were grouped around considerations on posture and mechanics in movement, rather than specifically on function or social context, but overall attempted to retain coarse representations of relevant behavioural states that would be useful in further investigations of behaviour using accelerometers. It is also important to note that accelerometers are incapable of measuring the costs of lactation directly as they remain blind to any physiological changes that may occur during a period of rest, despite any appreciable effects this may have on total energy expenditure. Any and all references to Presenting/Nursing-type behaviours as a result are only considered estimates of the behavioural aspects of lactation, rather than a measure of discrete energy transfer (Cameron 1998). All behavioural classification levels are summarized in **Table 2.2**.

Table 2.2: Summary of behavioural classification scheme used to classify different levels of behavioural resolution in classifying acceleration from behavioural states defined in section **2.5.1**. A summary of activity budgets as % of time in each behaviour (standard deviation) are also included for the two years that videos were decoded for **Chapter 3**.

						Activity Budget (%)	
Behaviour	BCS01	BCScoa	BCSmed	BCSfine	BCScon	2015	2016
[A]Rest	0	0	0	0	0	54.9 (26.85)	56.5 (26.65)
[A]Alert, head up	1	2	1	10	1	6.9 (5.71)	5.4 (6.11)
[A]Alert, head down	0	2	0	0	2	13.1 (12.10)	11.7 (11.15)
[A]Alert, head scan	1	2	1	10	3	4.7 (6.43)	5.1 (5.60)
[A]Pup Check	1	2	1	10	4	3.2 (2.62)	2.3 (1.79)
[A]Loco-motion	1	5	2	3	5	1.1 (2.77)	0.9 (2.06)
[A]Roll	1	6	3	14	6	0.1 (0.34)	0.3 (0.46)
[A]Comfort Movement	1	6	3	14	7	5.5 (3.76)	5.5 (3.31)
[A]Nose Pup	1	4	7	13	8	0.6 (1.02)	0.8 (1.81)
[A]Flippering Pup	1	6	4	8	9	1.3 (3.59)	1.6 (4.31)
[A]Mum-pup interaction	1	3	1	4	10	0.3 (1.62)	0.3 (1.49)
[A]Presenting /Nursing	1	4	5	6	12	4.3 (8.18)	5.5 (10.60)
[A]Presenting /Nursing, alert	1	4	5	6	13	1.7 (3.44)	1.5 (3.39)
[A]Nose Female	1	1	7	1	14	0.0 (0.02)	0.0 (0.01)
[A]Nose Male	1	1	6	2	15	0.0 (0.01)	0.0 (0.03)
[A]Nose Other	1	1	7	3	16	0.0 (0.21)	0.0 (0.06)

Table 2.2 con't: Summary of behavioural classification scheme used to classify different levels of behavioural resolution in classifying acceleration from behavioural states defined in section **2.5.1**. A summary of activity budgets as % of time in each behaviour (standard deviation) are also included for the two years that videos were decoded for **Chapter 3**.

						Activity Budget (%)	
Behaviour	BCS01	BCScoa	BCSmed	BCSfine	BCScon	2015	2016
[B]Open Mouth Threat, female	1	1	7	1	17	0.4 (0.84)	0.8 (2.01)
[B]Open Mouth Threat, male	1	1	6	2	17	0.5 (2.16)	0.3 (1.03)
[B]Open Mouth Threat, other	1	1	7	3	17	0.0 (0.09)	0.0 (0.16)
[B]Flipper Aggressive, female	1	1	7	1	18	0.0 (0.11)	0.0 (0.22)
[B]Flipper Aggressive, male	1	1	6	2	18	0.0 (0.03)	0.0 (0.33)
[B]Flipper Aggressive, other	1	1	7	3	18	0.0 (0.02)	0.0 (0.05)
[B]Slap	1	1	7	9	19	0.0 (0.03)	0.0 (0.18)
[B]Flipper Ground	1	1	7	12	20	0.1 (0.69)	0.0 (0.14)
[B]Flee	1	5	2	7	21	0.0 (0.01)	0.0 (0.05)
[B]Chase	1	5	2	7	22	0.0 (0.08)	0.0 (0.14)
[B]Bite, female	1	1	7	1	23	0.0 (0.06)	0.1 (0.23)
[B]Bite, male	1	1	6	2	23	0.0 (0.31)	0.0 (0.07)
[B]Bite, other	1	1	7	3	23	0.0 (0.07)	0.0 (0.04)
[B]Fight, female	1	1	7	1	24	0.0 (0.04)	0.1 (0.21)
[B]Fight, male	1	1	6	2	24	0.0 (0.13)	0.0 (0.01)
[B]Fight, other	1	1	7	3	24	-	-
[C]Attempted Copulation	1	8	9	11	25	-	-
[C]Copulation	1	8	9	11	25	-	0.1 (1.14)
[D]OTHER	1	3	8	5	26	0.3 (1.71)	0.4 (1.21)

2.6 – Components and derivations of acceleration

Data from accelerometers were downloaded upon retrieval using the AxyManager2 software (TechnoSmart Europe, Italy) and converted from their raw form to a comma delimited file for analysis, containing the sample timestamp and three separate raw acceleration vectors at each sample. Each accelerometer data file was examined using R (version 3.5.0; R Core Team 2018) to check formatting and was adjusted as necessary. For the purposes of deriving activity budgets from accelerometers, three hours of sampling time were removed from the start and end of the accelerometry data in order to account for any bias associated with post-tagging recovery and recapture, respectively, where recovery from sedation and human interaction may alter behaviour. In order to adequately summarize the acceleration signal, 33 attributes were derived and labeled as follows (**1-33**; see Shuert et al. 2018). Static acceleration (**1-3**), the gravitational component indicating position and posture in each axis of movement, was calculated using a moving average filter over a 3 second, or 150 data points when sampled at 50 Hz (Taylor 1994; Wilson et al. 2006; Shepard et al. 2008; Skinner et al. 2010; McClune et al. 2014). The dynamic component of acceleration (**4-6**), the component due to the movement of the individual, was then calculated by subtracting the static component from the raw acceleration in each axis (Wilson et al. 2006; Shepard et al. 2008; Bidder et al. 2014; McClune et al. 2014). Partial dynamic body acceleration (PBDA; **7-9**) was calculated as the absolute value of dynamic movement in each axis of movement (Green et al. 2009b; Fossette et al. 2010; Elliott et al. 2012; Ladds et al. 2016). Overall dynamic body acceleration (ODBA; **10**) and vectorial dynamic body acceleration (VeDBA; **11**) were also calculated as outlined below in **Eq. 2-1** and **2-2** (Gleiss et al. 2011; Gómez Laich et al. 2011).

$$ODBA = |dyX| + |dyY| + |dyZ| \quad \textbf{(Eq. 2-1)}$$

$$VeDBA = \sqrt{dyX^2 + dyY^2 + dyZ^2} \quad \textbf{(Eq. 2-2)}$$

A smoothed vector of VeDBA (VeDBAs; **12**) was also calculated as a 3-second running mean as with static acceleration (Gleiss et al. 2011; Gómez Laich et al. 2011; Qasem et al. 2012; Hicks et al. 2017). The ratio of VeDBA to PBDA was also included to indicate the relative contribution of each axis of PBDA to the vector of movement (**13-15**; Fehlmann et al. 2017). The change in acceleration over time, the third derivative of position commonly referred to as jerk, was derived by taking the differential of each axis of acceleration (**16-18**), in addition to the norm of jerk (**19; Eq. 2-3**) calculated by taking the square root of the sum of the squared differential of acceleration in each dimension:

$$norm\ jerk = f_s * \sqrt{\sum diff(A)^2} \qquad \textbf{(Eq. 2-3)}$$

where *fs* is the sampling frequency in Hz and *A* is all three axes of acceleration as outlined in Ydesen et al. (2014). Pitch and roll in radians were calculated as the arc sine of static acceleration in the heave (dorso-ventral movement) and sway (lateral movement) axes, respectively (**20-21**; Shepard et al. 2008). In order to match the resolution of behavioural sampling derived from video footage, these 21 feature variables were collapsed into 1-second discrete data windows (e.g. 50 samples wide at 50 Hz sampling rate) and summarized as a mean.

To characterize oscillations in dynamic body movement, elements of power spectral density and frequency were also calculated for each second of acceleration data in a window using Fourier analysis (Fehlmann et al. 2017). A fast Fourier transform (fft) decomposes an acceleration signal and translates it from a time-domain signal to a stationary frequency-domain signal whereby elements of frequency and power (amplitude) can be extracted (Yost et al. 1983). In order to avoid potential issues of spectral leakage (as a traditional Fourier analysis assumes that the signal continues on indefinitely) while including enough data to sample like locomotion, spectral elements were calculated over a window spanning 1 second on either side

of the window at hand (Yost et al. 1983). To summarize the windows, the first two maximum power spectral density peaks (PSD) were extracted along with their associated frequencies in each axis of movement (**22-33**; e.g. Fehlmann et al. 2017). Accelerometry feature variables with references can be found summarized in **Table 2.3** and adapted in Shuert et al. (2018). All accelerometry features were calculated and derived using custom R scripts, partially adapted from Fehlmann et al. (2017) in R version 3.5.0 (R Core Team 2018).

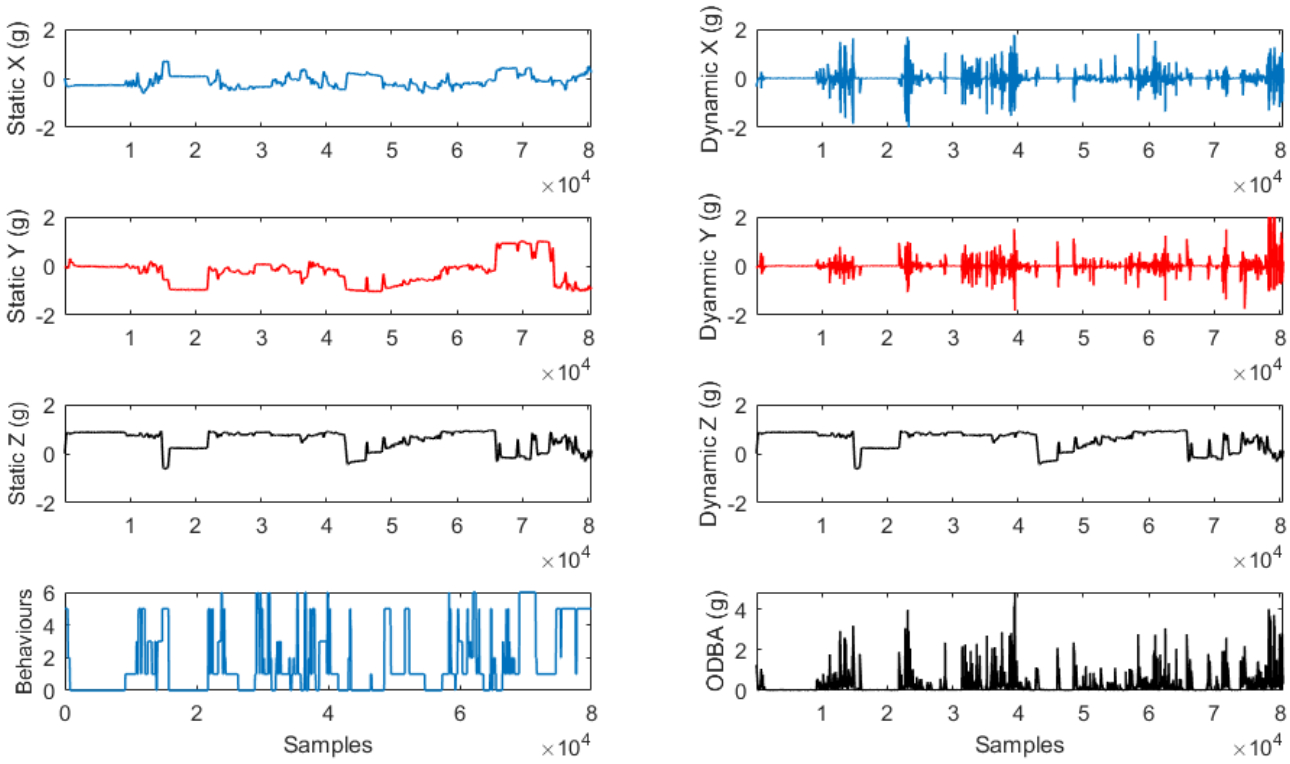


Figure 2.5: Example section of accelerometry data (50 Hz) with basic features including static and dynamic acceleration in each axis of movement as well as overall dynamic body acceleration (ODBA) over a 25 minute period of a female grey seal during lactation. Behaviours are also included as would be classified from BSCcoa (0: Rest, 1: Sociality/Aggression, 2: Alert, 3: Other, 4: Presenting/Nursing, 5: Locomotion, 6: Comfort Movements).

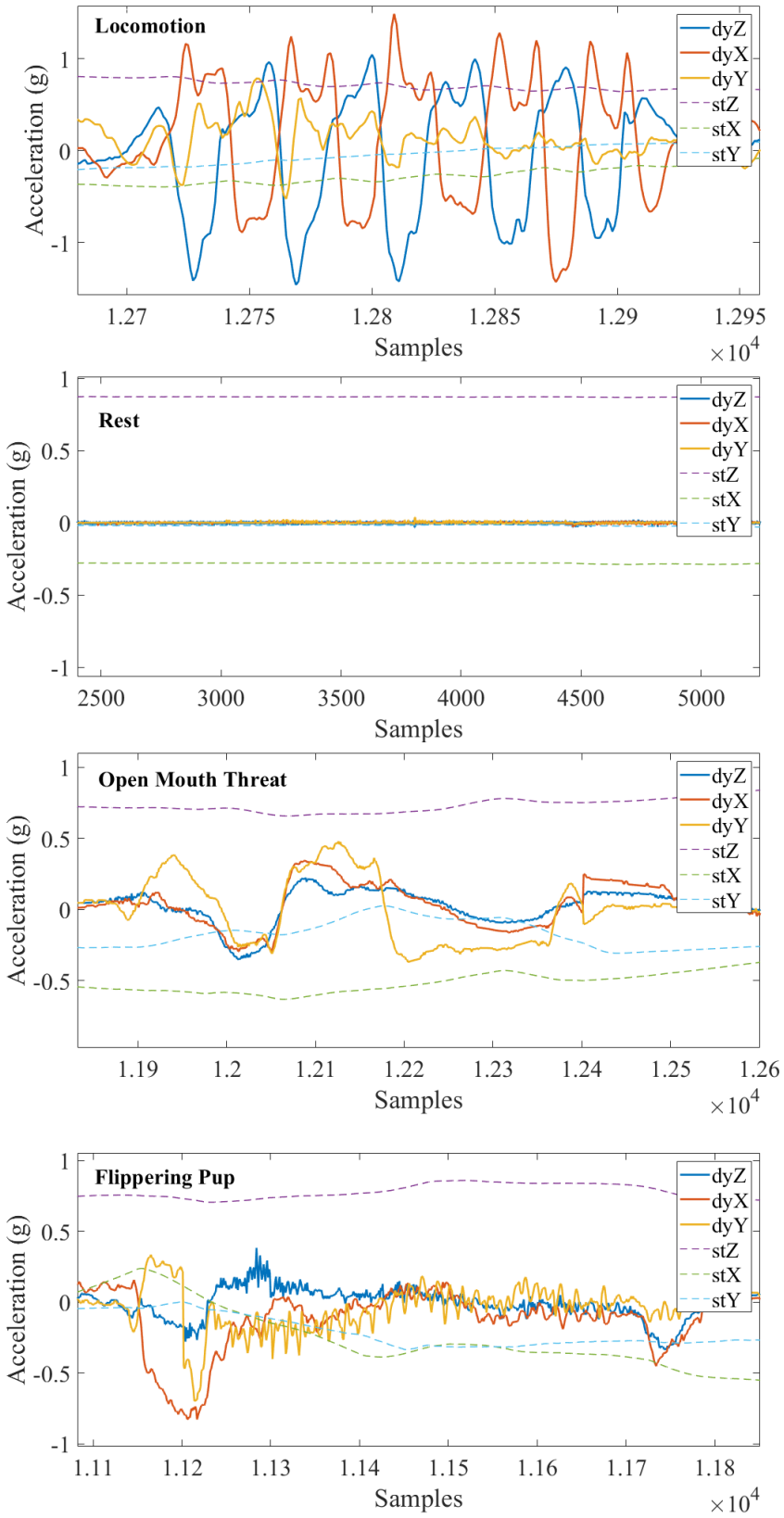


Figure 2.6: Example sections of behaviours from acclerometry data. Each behaviour (Locomotion, **A.3-LOCO**; Rest, **A.1-REST**; Open Mouth Threat, **B.1-OMT**, and Flippering Pup, **A.4-FP**) is summarized by static (stX, stY, stZ) and dynamic acceleration (dyX, dyY, dyZ, see **Table 2.3**) across varying lengths of time (50 Hz, samples).

Table 2.3: Summary of accelerometry feature variables as adapted and recently published with a manuscript summarizing the results from **Chapter 3** (Shuert et al. 2018). All feature variables, with the exception of those relating to power spectrum density and frequency (e.g. PSD1x, Freq1x), were summarized according to their mean value over 1 s, while those relating to Power spectrum and frequency elements (PSD1x – Freq2z) were derived over a 3 s moving window (1 s overlap on either side) to minimize spectral leakage (see section **2.5.1**).

No.	Feature Code	Description	References
1-3	stX, stY, stZ	Static acceleration (body posture) in each separate axis	(Taylor 1994; Wilson et al. 2006; Shepard et al. 2008; Skinner et al. 2010)
4-6	dyX, dyY, dyZ	Dynamic acceleration in each separate axis	(Wilson et al. 2006; Shepard et al. 2008; Bidder et al. 2014; McClune et al. 2014)
7-9	PBDAx, PBDAy, PBDAz	Partial dynamic body acceleration (absolute acceleration in each axis)	(Green et al. 2009b; Fossette et al. 2010; Ladds et al. 2016)
10	ODBA	Overall dynamic body acceleration	(Gleiss et al. 2011; Gómez Laich et al. 2011)
11, 12	VeDBA, VeDBAs	Vectorial dynamic body acceleration, smoothed	
13-15	ratioX, ratioY, ratioZ	Ratio of VeDBA to PDBA	(Fehlmann et al. 2017)
16-18	jerkX, jerkY, jerkZ	Jerk, derivative of acceleration, in each separate axis	(Ydesen et al. 2014)
19	jerkN	Norm of jerk in all axes	
20, 21	Pitch, Roll	Pitch and roll in radians	(Shepard et al. 2008)
22-24	PSD1x, PSD1y, PSD1z	Primary dominant power spectrum density in each axis	(Yost et al. 1983; Fehlmann et al. 2017)
25-27	PSD2x, PSD2y, PSD2z	Secondary dominant power spectrum density in each axis	
28-30	Freq1x, Freq1y, Freq1z	Frequency corresponding to the primary dominant power spectrum density in each axis	
31-33	Freq2x, Freq2y, Freq2z	Frequency corresponding to the secondary dominant power spectrum density in each axis	

2.7 – Energetics

Maternal post-partum mass (MPPM) was estimated by first calculating the difference in mass between the final and initial handling events (kg d^{-1}). This rate of mass loss was then used to back calculate an estimate of mass at birth based on the day of lactation upon the initial capture event. As each female's arrival on the colony was closely monitored to within a day, individual birth dates could be accurately monitored. In addition, the mass-specific rate of mass loss (mdml, g kg^{-1} d^{-1}) was calculated as the rate of mass lost divided by maternal post-partum mass as larger females tend to lose mass more quickly than smaller females (Wheatley et al. 2006). As a proxy for each female's energy usage over the lactation period, mass transfer efficiency (MT_{eff}) was calculated as the ratio of pup mass gain to female mass loss between the early lactation (tag deployment) and late lactation (tag retrieval) sampling events, expressed as a percentage (Iverson et al. 1993; Mellish et al. 1999a; Pomeroy et al. 1999);

$$\frac{Pup\ mass\ gain\ (kg)}{Female\ mass\ loss\ (kg)} \times 100 \qquad \textbf{(Eq. 2-4)}$$

Movement proxies like ODBA (**Eq. 2-1**) and VeDBA (**Eq. 2-2**) have been shown to track well in validation studies to more direct measures of metabolic rate such as heart rate, respirometry, and doubly-labelled water in a variety of species, from invertebrates to marine mammals and terrestrial carnivores during periods of exercise (Wilson and McMahon 2006; Fahlman et al. 2008; Green et al. 2009b; Halsey et al. 2009b, 2011a; Gleiss et al. 2011; Gómez Laich et al. 2011; Lyons et al. 2013; Wright et al. 2014; Miwa et al. 2015). It should be noted that ODBA and VeDBA have not been validated for grey seals and no equations exist to link these proxies directly to oxygen consumption rate. When calculated from accelerometry data located at the approximate centre of mass (torso-mounted accelerometers), ODBA and VeDBA have the potential to a be proxy measure of daily energetic expenditure (DEE) and have previously been partitioned into activity-specific energetic expenditure (AEE; Portugal et al.

2016). However, these movement proxies do not take into account basal condition and metabolism which may vary greatly between individuals (Wilson et al. 2006; Stothart et al. 2016); metabolism can vary significantly between individuals as a result of a variety of health, dominance, personality, and foraging conditions (e.g. Turbill et al. 2013; Stuber et al. 2015; Binder et al. 2016; Goulet et al. 2017). Alternatively, heart rate has been used extensively to get accurate qualitative and, if properly calibrated, quantitative measures of DEE, AEE, and background energy expenditure (e.g. metabolism) in a wide variety of taxa (Portugal et al. 2016; Halsey et al. 2019). Minimum heart rate (min-f_H) can be derived as a robust proxy of basal metabolic rate, while mean heart rate (mean-f_H) can be used as an estimate of DEE (Portugal et al. 2016). Activity-specific heart rate (also termed auxiliary heart rate; aux-f_H) can then be determined by subtracting min-f_H from the mean-f_H and used as proxy for AEE (Halsey et al. 2019). While this thesis would have ideally undertaken validation for either accelerometry- or heart rate-based energetics through such tools as doubly-labelled water in the field, cost and some ethical concerns prevented this.

2.8 – Environmental variables

Weather data for the 2015 season were gathered from a local weather station based in Anstruther, Fife (about 5.5 miles NE of the Isle of May, via wunderground.com) with 4 time points of temperature, precipitation (mm rain), and wind (mph) throughout each day. Weather data for the 2016 season were collected using a portable weather station that included a rain gauge, wind tachometer with directional magnetometer, and a temperature, humidity and pressure gauge (TFA Nexus, Germany), located at the centre of the island (see **Figure 2.2b** and **c**). Weather data for the 2017 season were collected again using the TFA Nexus system, but without the wind tachometer. In addition to the central weather station, 8 individual thermologgers (iButton, DS1921G-F5 thermochrons, HomeChip, UK) were housed in plastic cases and attached to stakes. Each stake was hammered into the ground around well-used

breeding sites on the colony in order to document the heterogeneity of surface temperatures experienced across the island. Each thermologger was set to record temperature every half hour for the duration of the 2017 breeding season (± 0.5°C see **Figure 2.2c**, **Figure 5.10**). Because of the disparity between years for available local weather data, the daily maximum temperature, total daily precipitation, and mean wind speed for each day were used to quantify the effect of environmental variables on grey seal behaviour in **Chapter 4**. Temperature data for 2015 and 2016 included in **Figure 2.5** below, relative to the maximum temperature measured in 2017 (**Figure 5.10**). Temperature data from iButtons were included for fine-scale analyses of the influence of external pressures on energetics for **Chapter 5**.

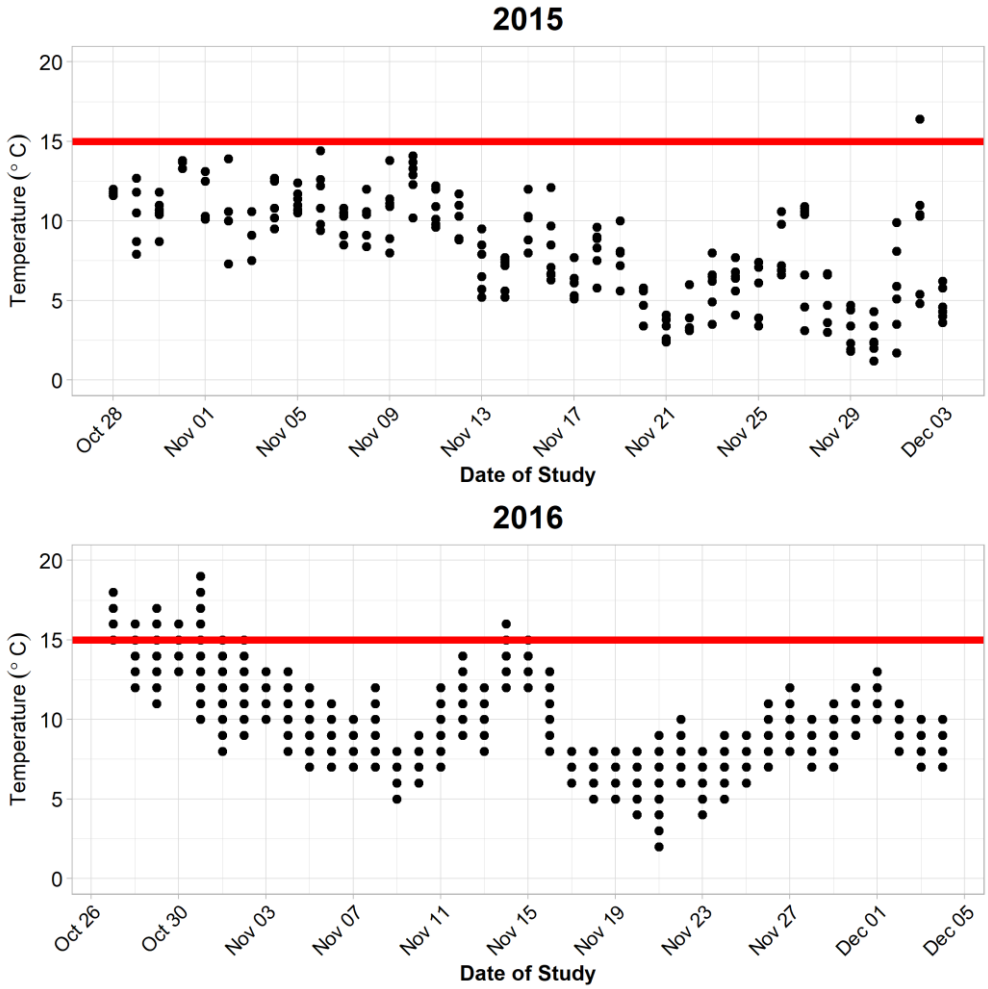


Figure 2.7: Temperature data from 2015 and 2016 relative to maximum temperature measured in 2017 (red line), extracted from a weather station located in coastal Anstruther for 2015 and from a weather station installed on the Isle of May for 2016. The 2016 season was the warmest included in this study.

Chapter 3:

Assessing the use of accelerometers and machine learning approaches to classify behaviour in lactating grey seals (*Halichoerus grypus*)

Manuscripts from this Chapter:

SHUERT, C. R., P. P. POMEROY, AND S. D. TWISS. 2018. Assessing the utility and limitations of accelerometers and machine learning approaches in classifying behaviour during lactation in a phocid seal. Animal Biotelemetry 6:14. [*Author Contributions*: CRS, SDT, and PPP conceived the study. CRS collected the data and performed the analyses with support from SDT and PPP. CRS wrote the paper with input from all co-authors.]

3.0 - Abstract

Classifying behaviour through the use of animal-borne accelerometers has quickly become a popular tool for remotely observing behavioural states in a wide variety of species. Most accelerometry work in pinnipeds has focused on at-sea behaviour, with no effort to date focusing on classifying behaviour in the brief, but important terrestrial phase of lactation and reproduction. As a result, this chapter sought to determine the best method for classifying behaviour by comparing two commonly used machine learning techniques and to what resolution of detail regarding behavioural state could be achieved using wild, free-ranging female grey seals. This chapter also investigated considerations for device placement, sampling rate, and individual consistency in movement for the behavioural states considered here in grey seals. Video footage was decoded using a high resolution behavioural ethogram that was collapsible into increasingly coarse resolution behavioural states and used to label concurrent sections of accelerometry data and used to train two sets of classification algorithms. In all, Random Forest classification models out-performed *k*-Nearest Neighbours across all levels of behavioural resolution. The best model was able to classify a coarse-resolution of behaviour reliably, including behaviours such as Resting, Alert, Presenting/Nursing, and a form of pup interaction, Flippering Pup, which represent the majority of a female grey seal's activity budget. Some rare and context dependent behaviours were not well captured. Device placement and sampling rate also played a significant role on the accuracy of behavioural classification. While there was significant variation between individuals in behavioural mechanics, individuals did not differ significantly within themselves. Using accelerometers to classify behaviour during lactation allows for a fine-scale assessment of time and energy trade-offs for species with fixed energy stores and could possibly be extended to other capital breeding pinnipeds.

3.1 – Introduction

Observing animals has been the hallmark approach of ethological studies. Often credited with formalizing the field, Altmann (1974) gave researchers a toolkit for sampling behaviour state and context in the field through comparable and repeatable approaches to measures of activity and interaction. Quantitative observational studies have been used to understand a wide range of behavioural contexts, such as individual- or population-level foraging decisions (Altmann 1974; Anderson and Harwood 1985; Witter et al. 2012) and investigating the mechanisms for conflict and conflict-avoidance (Bishop et al. 2014). Comparative observations also allow the examination of how behaviour may vary over time such as differences between day and night activity (Anderson 1978; Culloch et al. 2016) or across individuals, including personality types and consistent individual differences (McGhee and Travis 2010; Twiss and Franklin 2010; Briffa and Greenaway 2011). With the advancement of animal-borne data loggers such as accelerometers and time-depth recorders, researchers have been able to extend the application of behavioural studies to species that have typically been very difficult to observe in the wild, such as marine mammals (DeRuiter et al. 2013; Ydesen et al. 2014; Owen et al. 2016; Shorter et al. 2017). More specifically, tri-axial accelerometers have been used to remotely observe and infer animal behaviour in pinnipeds when they are unobservable during trips to and from feeding aggregations (Yeates et al. 2007; Davis et al. 2013; Battaile et al. 2015; Arthur et al. 2016; Jeanniard-du-dot et al. 2017) and other diving patterns and at-sea behaviour (McClintock et al. 2013; Jeanniard-du-dot et al. 2016). Often, these accelerometry deployments focus on building coarse-scale activity budgets for resolving energetics associated with foraging and diving or towards event detection, such as head-striking behaviour, to infer the rate of prey consumption relative to energy expenditure at sea (Skinner et al. 2010; Viviant et al. 2010; Watanabe and Takahashi 2013; Ydesen et al. 2014). These studies tend to focus on species who exhibit an income approach to the

reproductive period of their life history, in which they must regularly supplement their energy stores to maintain and provision their pups, or instead focus on detecting and classifying behaviour outside of the reproductive period (Arthur et al. 2016; Jeanniard-du-dot et al. 2017). While accelerometers have been used extensively to study the behaviour of terrestrial animals, rarely has any research been geared to the consequences of behaviour associated with the brief, but important on-land portion of seal life history (e.g. Moreau et al. 2009; Soltis et al. 2012; McClune et al. 2014; Lush et al. 2015; Fehlmann et al. 2017; Pagano et al. 2017).

As with the development of accelerometers, the application of machine learning methods has also become a popular tool for remotely classifying behaviour from accelerometers in a variety of species (e.g. Hokkanen et al. 2011; Grünewälder et al. 2012; Joseph et al. 2017; Ladds et al. 2017). While accelerometers often present a novel tool for capturing behaviour, the associated data sets can quickly become monumental tasks to examine by hand (Brown et al. 2013). Supervised machine learning presents a way to overcome this. By using a period of time where the behaviour of an individual is known, a concurrent set of accelerometry data can be labelled and used to train a classification algorithm of choice in order to classify behaviour outside of the observable period (Brown et al. 2013). Many different algorithms are available to use in classification, ranging from simple linear discriminant analyses (e.g. Nathan et al. 2012) and decision tree algorithms (e.g. Nishizawa et al. 2013), to more advanced black-box type approaches such as random forests (e.g. Lush et al. 2015), support vector machines (e.g. Hokkanen et al. 2011), and artificial neural networks (e.g. Banerjee et al. 2012). Gaining access to individuals in order to build a training dataset can often be challenging. Captive surrogates have been used with accelerometers mounted in an analogous way to those in the wild and used to train an algorithm to classify the behaviour of their wild counterparts (e.g. Soltis et al. 2012; Campbell et al. 2013; Dalton et al. 2014; McClune et al. 2014). One such study noted, however, that captive surrogates may not exhibit

behaviour in the same mechanistic fashion as those in the wild which may lead to poor, yet undetectable, model performance in classification of unknown data in wild individuals (Pagano et al. 2017). Having access to behavioural information in a wild context is therefore key to ensuring that trained data match that of a wild cohort of individuals and will likely more accurately characterize behaviour when out of sight.

During their 18-day period on shore, breeding female grey seals have a fixed energy store that they must allocate to maintain themselves and their pup (Kovacs 1987; Pomeroy et al. 1994; Lydersen and Kovacs 1999). Behavioural decisions and small fluctuations in activity likely have an impact on this resource allocation as the margin of error for expending energy reserves is minimal. Grey seals offer a good system to examine activity in detail, but visual observations to assess behaviour are limited to daylight hours. During the grey seal breeding season in the autumn, this may only be about one third of their daily cycle at best. Developing a species-specific supervised machine learning algorithm would be extremely powerful in order to elucidate behaviour outside of this limited observable time and record behaviour on a much finer-scale than previously accessible through traditional observation approaches. While many previous studies have evaluated the mechanics of behaviour at sea (e.g. Ladds et al. 2016), it is unclear if any efforts have been made to resolve and classify lactation behaviour on land in grey seals using accelerometry. Accelerometry-derived activity will also allow for the assessment not only of behaviour overnight, an area of research that is largely either ignored or inaccessible (Culloch et al. 2016), but also will overcome the limitations of visual focal sampling by recording data continuously and simultaneously over many individuals, free from observer biases.

In order to conserve energy resources, grey seals tend to remain inactive for long periods of time and only move about to either reposition themselves relative to a pup or to

intercept a threat, be it a male or another female (Kovacs 1987). Grey seals are also known to occasionally travel to and from pools for thermoregulation, though the cost of this is largely unevaluated (Twiss et al. 2002; Stewart et al. 2014). Most active behaviours are therefore limited to those such as vigilance or pup-checking where the head may be in motion, leaving the body largely unmoving. Consistent individual differences in time spent alert has already been shown as an important indicator of stress management and coping styles in grey seals (Twiss and Franklin 2010; Twiss et al. 2012b; a). While many studies advise placement of accelerometers close to the centre of mass as a better indicator of energy expenditure (e.g. Brown et al. 2013), head-mounted accelerometers may give a better indication of vigilance, an important indicator of stress management in many terrestrial animals (Caro 1987; Burger and Gochfeld 1994; Arenz and Leger 1999; Pangle and Holekamp 2010; Yorzinski et al. 2015; Kölzsch et al. 2016). This motivated the comparison of the resolution of data from both head (vigilance) and torso-mounted accelerometers (energy expenditure) in the same context and evaluate trade-offs associated with behaviour detection for a largely inactive model species (**Figure 3.1**). This study encompassed two successive breeding seasons. Given that grey seals are site faithful (Pomeroy et al. 1994), this study also sought to attempt to quantify the amount of variability and repeatability between years for accelerometry feature characteristics measured in repeat capture females as well as the amount of variance between individuals. However, individuals are also exposed to varying environmental conditions and animal densities across years that may limit the repeatability of accelerometry data within individuals.

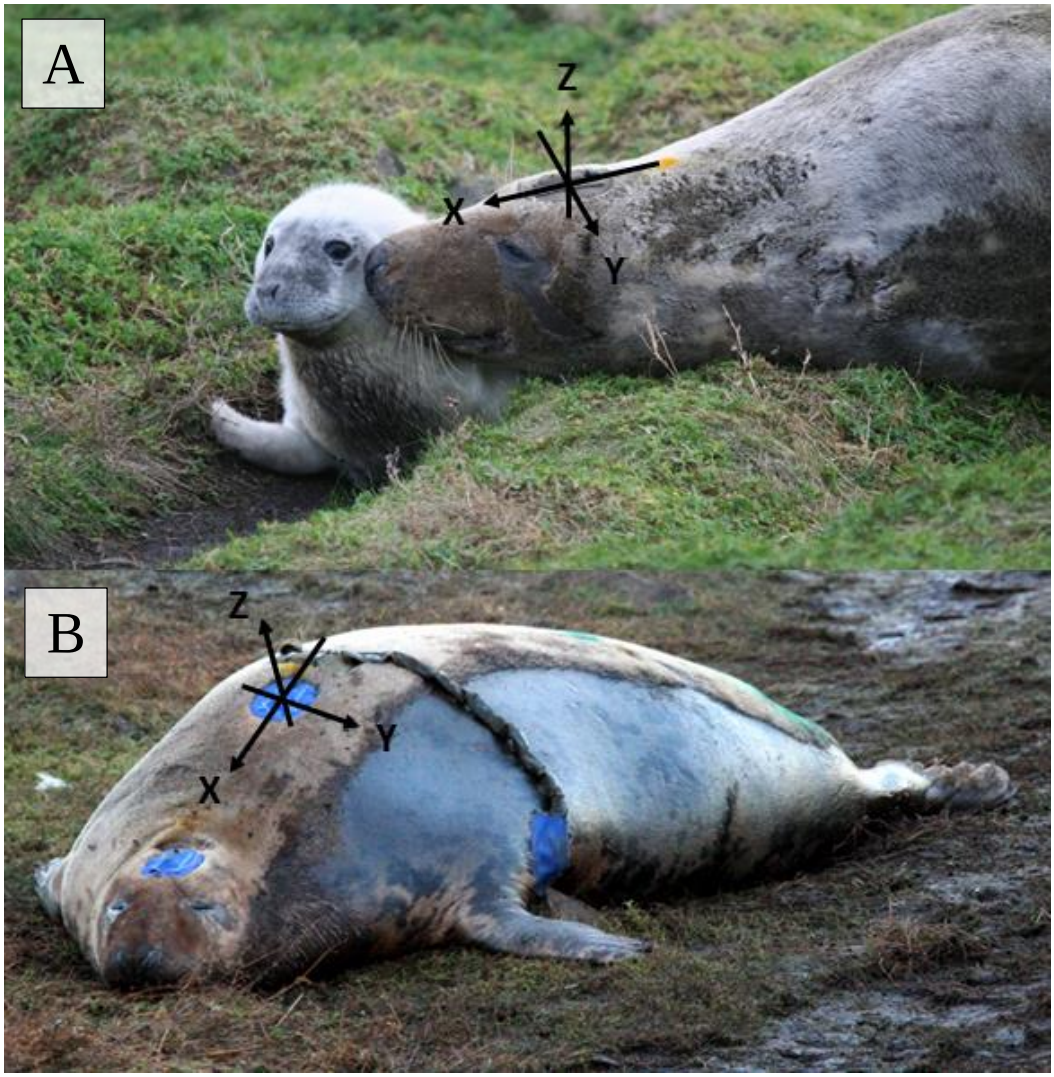


Figure 3.1: Example of attachment set up for (A) a head-mounted accelerometer, and (B) a torso-mounted accelerometer in addition to a head-mount, contained within a custom designed ballistic nylon footprint on a female grey seal. Tag-frame axes labelled with arrows pointing in direction of positive acceleration values for each axis (X, Y, Z). Each accelerometer was configured to measure ± 2g at 50 Hz (2015) and 25 Hz (2016). Heart rate monitor also pictured in panel B (Photos: CR Shuert).

The main aim of this chapter was to build a useable ethogram of behavioural states derived from accelerometers during lactation on several levels of behavioural resolution across two years of study. Video footage of female grey seals was decoded using a very detailed ethogram of behaviours that could be condensed into larger categories of states as part of a behavioural classification scheme and used to label concurrent accelerometer data (as detailed in **2.5.1** and **2.5.2**). Several females in 2016 were equipped with two accelerometers, one on the head and one on the torso, to evaluate the effect of placement on behavioural detection. In

order to evaluate the effectiveness of classification algorithms, a subset of labelled acceleration data (75%) were used to train a relatively simple classification algorithm, in this case a *k*-Nearest Neighbours (kNN) algorithm, as well as a more advanced random forest classification algorithm. Model performance for both the kNN and random forest models was then assessed using the remaining labelled acceleration data (25%). Model performance of these two algorithms was then compared across 4 levels of behavioural resolution ranging from detailed, context-specific behaviour to simple, broad active and inactive categorization (as detailed in section **2.5.3**). Behaviour detection associated with accelerometer placement was compared between accelerometers mounted on the head between both years of study and those torso using separately trained algorithms. As an evaluation of the consistency of behaviours, repeatability and variance estimates were bootstrapped across top accelerometry feature variables thought to best describe individual behaviours.

3.2 – Methods

3.2.1 – Derivation of accelerometry features

For the purposes of this analysis, 53 female grey seals were equipped with small data-logging accelerometers (AXY-Depth, TechnoSmart Europe, Italy) during the core of the lactation period (10.7 ± 2.7 days) for the 2015 and 2016 breeding seasons (as described in sections **2.2** and **2.3**; repeat captures between seasons, $n = 11$). Female body mass at deployment and recovery was used to calculate a linear daily mass loss and allowed for the estimation of maternal post-partum mass. All individuals during the 2015 and 2016 seasons were equipped with an accelerometer mounted on the head, while 10 individuals in the 2016 season were additionally equipped with an accelerometer on the torso, mounted roughly between the shoulder blades (**Figure 3.1**). Accelerometers were configured to sample at 50 and 25 Hz continuously for the length of deployment for the 2015 and 2016 seasons, respectively. Acceleration signals were processed to derive 33 separate attributes as described

in section **2.6** (Wilson et al. 2006; Shepard et al. 2008). These included static and dynamic acceleration (stX-Z and dyX-Z, respectively; acceleration attributes **2.6-1:6**), partial dynamic body acceleration (PBDAx-z; attributes **2.6-7:9**), ODBA (attribute **2.6-10**), VeDBA as well as VeDBAs (attributes **2.6-11:12**), the ratio of VeDBA to PBDA (ratioX-Z; attributes **2.6-13:15**), jerk (jerkX-Z; attribute **2.6-16:18**) and the norm of jerk (jerkN; attribute **2.6-19**), as well as pitch and roll (attributes **2.6-20:21**). These attributes were summarized by their mean over a 1-second window. To capture the frequency-domain aspects of movement, the primary and secondary dominant power spectrum densities and their corresponding frequencies (PSD1x-z, PSD2x-z, Freq1x-z, Freq2x-z; attributes **2.6-22:33**) were derived through Fourier analysis and calculated over a 3s window (1s on either side of the moment of interest). A summary list of feature variables and references used in this section can be found in **Table 2.3** (Shuert et al. 2018).

3.2.2 – Time-matched behaviours and datasets

Females were observed and recorded over the course of each deployment according to the protocol outlined in section **2.4**. All daytime video footage was decoded for all behaviours listed in **2.5.1** using the methods presented in **2.5.2**. Behaviours were collapsed into 4 separate behavioural classification scheme models for assessing levels of resolution as described in **2.5.3** and **Table 2.2** (BCS01, BCScoa, BCSmed, BCSfine). Briefly, BCS01 represents inactive behaviours (**5.2.1-A.1** and **A.2-ALHD**) against all others; BCScoa includes resting (0), social (1), alert (2), other (3), presenting/nursing (4), locomotion (5), and comfort movement (6) type behaviours; BCSmed includes behaviours pertaining to resting (0), alert (1), locomotion (2), comfort movement (3), flippering pup (4), presenting/nursing (5), male interaction (6), female interaction (7), and other (8); and finally, BCSfine includes resting (0), social and threat behaviours for females (1), or males separately (2), locomotion (3), female-pup interaction (4), other (5), presenting/nursing (6), chasing and fleeing (7), flippering pup (8), alert (10),

flippering the ground (12), nosing pup (13), and comfort movement (14) behaviours. Concurrent sections of summarized attributes of acceleration data were extracted and time-matched with these BCS-levels to create a set of training data for each year and tag attachment type. Labelled data for 2015 head-mounted accelerometers totalled approximately 45.7 hours (n_{ind} = 29 individuals), while 2016 head- and torso- mounted accelerometers totalled approximately 91.3 (n_{ind} = 24) and 65.7 hours (n_{ind} = 10), respectively. This meant an average of 7.36 ± 15.5 hours per behaviour across years. A summary of the proportion of behaviours sampled between 2015 and 2016 is included in **Table 2.2**. In order to test the efficacy of the machine learning algorithms, 75% of these labelled training data were used to build the model and 25% held-out for validation. A glitch in the firmware was detected for the 2015 data following deployment that resulted in an early shut off during recording of 1.5 days into the deployment of 11 individuals. While the early shut off means that these individuals could not be included in assessment of activity budgets in **Chapter 4**, any data that could be labelled from focal video footage taking place within the 1.5 days was still used in training. This same glitch also meant that sampling rate between years differed as previously mentioned.

3.2.3 – Comparing machine learning algorithms

3.2.3a – k-nearest neighbours

In order to determine the best method for classifying behaviours according to the 33 feature variables above, two different classification algorithms were considered. The first is a simple, but intuitive kNN classification algorithm. This type of machine learning algorithm can be used to classify new acceleration data relative to the position of training data in a multi-dimensional space (Bidder et al. 2014; McClune et al. 2014). First, the Euclidean distance in ordination space between the reserved, new data point (x_j) and each training data point (x_i) is calculated for each feature dimension (a_r):

$$d(x_i, x_j) = \sqrt{\sum_{r=1}^{n} (a_r(x_i) - a_r(x_j))^2} \quad \textbf{(Eq. 3-1)}$$

The algorithm then selects the *k* number of training points with the lowest Euclidean distance (nearest neighbours) and classifies the new point based on the majority vote of those values (Bidder et al. 2014). Data points were only classified if the proportion of winning votes (n_{wc}) to the number of neighbors (*k*) was greater than 0.25 (*prob*; at least 3 neighbours), as given by the equation below from Bidder et al. (2014).

$$prob = \frac{n_{wc}}{k} \quad \textbf{(Eq. 3-2)}$$

For the purposes of this analysis, *k* was set to 11. Models were trained separately at each level of the classification scheme (BCS01, BCScoa, BCSmed, BCSfine, **Table 2.2**), resulting in a total of 4 separate kNN models to compare the resolution of classification using the 'class' package in R (Venables and Ripley 2002; Bidder et al. 2014).

3.2.3b – Random forests

The random forest algorithm is a fairly recent development and extension of classification and regression trees (Cutler et al. 2007). Classification trees are typically built by assembling binary partitions along increasingly homogenous regions of features with respect to the desired classification (Breiman 1999). These homogeneous splits, referred to as nodes, are continuously subdivided until there is no longer a decrease in the Gini impurity index, *G* (or in this case, it will approach zero as a single behaviour is included in the node):

$$G = \sum_{i=1}^{n} p_i(1 - p_i) \quad \textbf{(Eq. 3-3)}$$

where *n* is the number of behavioural classes and p_i is the proportion of each class in a set of observations, defined as the probability of mislabelling a randomly drawn element from the

data in each node (Cutler et al. 2007). Random forest fits many of these classification trees to a data set, combining predictions from all trees to classify new data (Breiman 1999; Cutler et al. 2007; Fehlmann et al. 2017). First, a training dataset is sampled randomly with replacement, resulting in several bootstrapped samples. With each of these simulated datasets, the model grows one tree to classify the observations into different classes, or behaviours, by hierarchical decision making down each node (Breiman 1999; Cutler et al. 2007). This algorithm utilizes bootstrapped samples from the original dataset to grow each individual tree, using a random selection of predictor variables, or in this case accelerometry features (**3.2.1**), to partition the data. Out-of-bag observations (OOB), or rather those predictor variable observations not included in each bootstrapped sample, are then used to calculate model accuracies and error rates and then averaged across all trees. Random forests offer a great number of iterations (number of trees) and several layers of randomness in order to build a robust and powerful tool for classification of new data, while limiting overfitting and problems associated with unbalanced datasets, as we might find in a seal's activity budget where rest often dominates the activity budget (e.g. Kovacs 1987; Fehlmann et al. 2017). Random forests also have the advantage of allowing for the assessment of variable importance by way of subtracting the parent variable Gini index value relative to the next two subsequent Gini index values for each feature variable. For this machine learning algorithm, the data were again split into a 75/25% training and testing set and grew 500 trees using the 'randomForest' package in R (Liaw and Wiener 2002).

3.2.4 – Model performance of classification algorithms

In order to compare model performance in each of the machine learning algorithms used in this study, precision, recall, and the F1 statistic were calculated from the resulting confusion matrices produced from each of the cross-validations used with the testing datasets. These metrics were assessed from the resulting values of true positives (correctly classified

positive values, TP), false positives (incorrectly classified positive values, FP), and false negatives (incorrectly classified values that were negative, FN) for each behavioural category (Powers 2011). Following the definitions from Bidder et al. (2014), Precision, also referred to as the true positive accuracy, was defined as the proportion of positive behavioural classifications that were correct, and was calculated as:

$$Precision\ (P) = \frac{TP}{TP + FP} \quad \textbf{(Eq. 3-4)}$$

Recall, also known as sensitivity, was defined as the proportion of new data pertaining to behaviours that were correctly classified as positive, and was calculated as:

$$Recall\ (R) = \frac{TP}{TP + FN} \quad \textbf{(Eq. 3-5)}$$

The F1 statistic represents the harmonic mean of Precision and Recall and was used as a metric for overall performance of each behavioural classification category as it computes the harmonic mean of both performance metrics (Powers 2011). F1 was calculated as:

$$F1 = \frac{2}{\frac{1}{Precision} + \frac{1}{Recall}} \quad \textbf{(Eq. 3-6)}$$

Values closer to 1 for all three model metrics stated above represent better model performance. Model creation and validation were performed separately for the 2015 and 2016 season, as well as separately for head-mounted and torso-mounted accelerometers (2016 only) on all BCS-levels previously defined. Variable importance plots for the random forest models were also examined.

3.2.5 – Stereotypy of behaviour

Consistency of behaviours with respect to key feature variables was also assessed between seasons for repeat capture females (n_{ind} = 11), something that is rarely available in relation to individual variability of behaviours in non-captive individuals. In order to achieve

equivalent sampling rates between seasons, the 2015 accelerometry data were down-sampled by half when compared to the 2016 accelerometry data. Generalized linear mixed effects models were built to predict top feature variables deemed most relevant for separating behaviour. Individual ID was included as a random effect in the model. To account for potential changes in cost-of-transport between years, individual estimated post-partum masses were added as fixed effects in R (package 'nlme'; Pinheiro et al. 2014). Variance and repeatability estimates associated with individual ID and year were calculated using the 'rptR' package (Stoffel et al. 2017), calculated over 500 bootstrapped samples. As a result of the inclusion of a fixed effect in this model, all repeatability measures are adjusted-R (*adj*-R) as per Stoffel et al. (2017). Significance of repeatability was assessed through the use of likelihood ratio tests to compare to a model without the random effect within the R package.

3.3 – Results

Static behaviours such as Resting, Presenting/Nursing, Flippering pup, and others were among those most consistently classified well across all models (**Table 3.1a-d**). Overall, the coarse level of the behavioural classification scheme (BCScoa) was found to classify the greatest number of behavioural states with the highest average F1 across the greatest number of behaviours (**Table 3.1b**). As a result, the majority of results presented focus on this single BCScoa model for brevity. To ease discussion of these results, behaviours 0-6 of BCScoa will be referred to as (0) Resting, (1) Social/Aggression, (2) Alert, (3) Other, (4) Presenting/Nursing, (5) Locomotion, and (6) Comfort Movement. Most often, all other behaviours were misclassified as Resting between years and accelerometer placements (**Table 3.2a-c**). Random forest models as a whole outperformed kNN classification models on every BCS level tested. On average, random forest models increased F1 values by 0.07 ± 0.11 from the same models classified with kNN (**Table 3.1a-d**). Random forests consistently improved the precision of classification, but did little to improve the recall for most behavioural states

(**Figure 3.2**). Accelerometers sampling at a higher frequency (50 Hz in 2015) were able to classify behaviour better for behaviours such as those relating to Alert and Locomotion than those sampled at a lower frequency (25 Hz in 2016). Torso-mounted accelerometers, however, generally performed better than head-mounted accelerometers on many of the static behaviours, with the exception of alert-type behaviours (**2.5.1-A.2**; **Table 3.1a-d**). Locomotion events, however, were completely undetected in torso-mounted accelerometers (**Table 3.2c**). Error estimates and out-of-bag errors for all 4 BCS-levels plotted against number of trees grown can be found in the appendix (**Figure A3.1a-d**).

Table 3.1a: Precision (P), recall (R), and F1 of BCS01 for active (1) and inactive (0) behaviours as defined in **Table 2.2** modelled using kNN and random forest for 2015 head-mounted, 2016 head-mounted, and 2016 torso-mounted accelerometers.

		kNN			Random Forest		
	Behaviour	P	R	F1	P	R	F1
2015 Head	0/1	0.748	0.839	0.791	0.758	0.877	0.813
2016 Head	0/1	0.761	0.916	0.831	0.764	0.956	0.849
2016 Torso	0/1	0.765	0.922	0.836	0.789	0.952	0.863

Table 3.1b: Precision (P), recall (R), and F1 of BCScoa for resting (0), social (1), alert (2), other (3), presenting/nursing (4), locomotion (5), and comfort movement (6) behaviours as defined in **Table 2.2** modelled using kNN and random forest for 2015 head-mounted, 2016 head-mounted, and 2016 torso-mounted accelerometers.

		kNN			Random Forest		
	Behaviour	P	R	F1	P	R	F1
2015 Head	0	0.658	0.714	0.685	0.761	0.770	0.765
	1	0	0	0	1.000	0.011	0.022
	2	0.556	0.636	0.593	0.626	0.773	0.692
	3	0.889	0.032	0.062	0.923	0.048	0.092
	4	0.554	0.402	0.466	0.723	0.262	0.385
	5	0.353	0.010	0.020	0.900	0.030	0.059
	6	0.658	0.154	0.234	0.610	0.203	0.305
2016 Head	0	0.670	0.877	0.760	0.677	0.919	0.780
	1	0.262	0.013	0.025	0.412	0.014	0.027
	2	0.405	0.294	0.341	0.460	0.291	0.356
	3	0.159	0.006	0.011	1.000	0.004	0.007
	4	0.470	0.141	0.217	0.796	0.198	0.317
	5	0.323	0.010	0.020	0.400	0.008	0.016
	6	0.383	0.052	0.092	0.686	0.059	0.109
2016 Torso	0	0.664	0.887	0.760	0.718	0.930	0.810
	1	0.264	0.008	0.017	0.583	0.014	0.027
	2	0.395	0.242	0.300	0.577	0.404	0.475
	3	0.361	0.015	0.029	0.833	0.011	0.022
	4	0.498	0.253	0.335	0.747	0.452	0.563
	5	0	0	0	0	0	0
	6	0.350	0.057	0.098	0.603	0.071	0.127

Table 3.1c: Precision (P), recall (R), and F1 of BCSmed for resting (0), alert (1), locomotion (2), comfort movement (3), flippering pup (4), presenting/nursing (5), male interaction (6), female interaction (7), and other (8) behaviours as defined in **Table 2.2** modelled using kNN and random forest models 2015 head-mounted, 2016 head-mounted, and 2016 torso-mounted accelerometers.

		kNN			Random Forest		
	Behaviour	P	R	F1	P	R	F1
2015 Head	0	0.716	0.910	0.801	0.714	0.970	0.823
	1	0.353	0.199	0.254	0.510	0.132	0.209
	2	0.357	0.017	0.032	0.842	0.027	0.052
	3	0.219	0.018	0.033	0.517	0.016	0.031
	4	0.555	0.304	0.393	0.655	0.393	0.492
	5	0.564	0.419	0.481	0.752	0.535	0.625
	6	0	0	0	1.000	0.014	0.029
	7	0.143	0.002	0.004	0.667	0.004	0.009
	8	0.714	0.025	0.048	1.000	0.049	0.094
2016 Head	0	0.744	0.968	0.841	0.740	0.994	0.849
	1	0.257	0.065	0.104	0.342	0.017	0.034
	2	0.250	0.010	0.019	0.444	0.016	0.031
	3	0.176	0.007	0.014	0.647	0.004	0.009
	4	0.526	0.185	0.274	0.686	0.216	0.328
	5	0.486	0.143	0.222	0.835	0.198	0.321
	6	0.231	0.007	0.013	0	0	0
	7	0.204	0.009	0.018	0.308	0.007	0.014
	8	0.133	0.004	0.008	0	0	0
2016 Torso	0	0.748	0.969	0.844	0.761	0.990	0.860
	1	0.275	0.053	0.088	0.596	0.067	0.120
	2	0	0	0	0.500	0.005	0.010
	3	0.189	0.012	0.023	0.510	0.013	0.025
	4	0.500	0.204	0.290	0.672	0.273	0.388
	5	0.506	0.265	0.348	0.759	0.490	0.596
	6	0.667	0.008	0.017	0	0	0
	7	0.163	0.006	0.012	0.571	0.014	0.028
	8	0.167	0.001	0.003	1.000	0.005	0.011

Table 3.1d: Precision (P), recall (R), and F1 of BCSfine for resting (0), social and threat behaviours for females (1), and males (2), and locomotion (3), female-pup interaction (4), other (5), presenting/nursing (6), chasing and fleeing (7), flippering pup (8), alert (10), flippering the ground (12), nosing pup (13), and comfort movement (14) behaviours as defined in **Table 2.2** modelled using kNN and random forest for 2015 head-mounted, 2016 head-mounted, and 2016 torso-mounted accelerometers.

		kNN			Random Forest		
	Behaviour	P	R	F1	P	R	F1
2015 Head	0	0.615	0.790	0.692	0.665	0.871	0.754
	1	0	0	0	1.000	0.006	0.011
	2	0.334	0.260	0.292	0.527	0.370	0.434
	3	0.233	0.016	0.031	0.792	0.031	0.060
	4	1.000	0.022	0.043	1.000	0.043	0.083
	5	0.714	0.050	0.093	0.789	0.074	0.135
	6	0.530	0.455	0.490	0.686	0.599	0.639
	7	0	0	0	0	0	0
	8	0.531	0.310	0.391	0.637	0.414	0.502
	10	0.330	0.325	0.327	0.420	0.428	0.424
	12	0	0	0	0	0	0
	13	0	0	0	1.000	0.004	0.008
	14	0.203	0.030	0.049	0.443	0.025	0.046
2016 Head	0	0.654	0.926	0.767	0.637	0.985	0.774
	1	0.221	0.014	0.026	0.429	0.017	0.032
	2	0.204	0.073	0.107	0.314	0.012	0.023
	3	0.234	0.015	0.028	0.444	0.016	0.031
	4	0	0	0	0	0	0
	5	0.130	0.006	0.012	1.000	0.004	0.008
	6	0.464	0.153	0.230	0.800	0.217	0.342
	7	0	0	0	0	0	0
	8	0.508	0.194	0.281	0.655	0.230	0.340
	10	0.246	0.122	0.163	0.335	0.072	0.119
	12	0	0	0	0	0	0
	13	0	0	0	0	0	0
	14	0.141	0.010	0.018	0.545	0.005	0.010
2016 Torso	0	0.651	0.930	0.766	0.672	0.976	0.796
	1	0.111	0.005	0.010	0.642	0.025	0.047
	2	0.234	0.080	0.119	0.557	0.148	0.234
	3	0	0	0	0.500	0.005	0.010
	4	0.345	0.074	0.122	0.500	0.028	0.054
	5	0.167	0.004	0.008	1.000	0.013	0.026
	6	0.496	0.279	0.357	0.745	0.507	0.604
	7	0	0	0	0	0	0
	8	0.462	0.206	0.285	0.703	0.302	0.422
	10	0.244	0.087	0.128	0.512	0.144	0.225
	12	0	0	0	0	0	0
	13	0.500	0.005	0.010	0	0	0
	14	0.169	0.019	0.034	0.462	0.015	0.029

Table 3.2: Confusion matrix showing the predicted behaviours (rows; (a) originating from the KNN classification for head-mounted accelerometers, (b) from random forest (RF) classification for head-mounted accelerometers, or (c) from RF classification for torso-mounted accelerometers) and against the observed reference behaviours (columns; originating from the focal video data) for model BCScoa for accelerometers mounted on the head. Behaviours included rest (0), social and aggression (1), alert (2), other (3), presenting/nursing (4), locomotion (5), and comfort movement (6). Values across the diagonal (**bold**) represent those behaviours that were correctly identified, true positives.

(a) Head-mounted Accelerometer

		Reference							
		0	1	2	3	4	5	6	Tot. Pred.
Prediction (kNN)	0	**13879**	*66*	*5184*	*80*	*783*	*137*	*976*	21105
	1	*0*	**0**	*1*	*0*	*1*	*0*	*0*	2
	2	*5003*	*281*	**9774**	*155*	*791*	*435*	*1151*	17590
	3	*0*	*0*	*1*	**8**	*0*	*0*	*0*	9
	4	*399*	*7*	*283*	*1*	**1172**	*9*	*244*	2115
	5	*1*	*1*	*6*	*0*	*0*	**6**	*3*	17
	6	*163*	*6*	*122*	*5*	*167*	*5*	**433**	901
Tot. Obs.		19445	361	15371	249	2914	592	2807	**41739**

(b) Head-Mounted Accelerometer

Prediction (RF)	0	**14976**	*32*	*3211*	*55*	*519*	*73*	*824*	19690
	1	*0*	**4**	*0*	*0*	*0*	*0*	*0*	4
	2	*4185*	*312*	**11876**	*176*	*701*	*488*	*1231*	18969
	3	*0*	*0*	*1*	**12**	*0*	*0*	*0*	13
	4	*188*	*8*	*196*	*2*	**1527**	*9*	*181*	2111
	5	*0*	*0*	*0*	*0*	*1*	**18**	*1*	20
	6	*97*	*5*	*89*	*4*	*166*	*4*	**570**	935
Tot. Obs.		19446	361	15373	249	2914	592	2807	**41742**

(c) Torso-mounted Accelerometer

Prediction (RF)	0	**20405**	*303*	*4708*	*290*	*917*	*114*	*1684*	28421
	1	*4*	**7**	*1*	*0*	*0*	*0*	*0*	12
	2	*1370*	*185*	**3265**	*147*	*225*	*85*	*380*	5657
	3	*0*	*0*	*0*	**5**	*1*	*0*	*0*	6
	4	*131*	*1*	*77*	*1*	**975**	*3*	*117*	1305
	5	*0*	*0*	*0*	*0*	*0*	**0**	*0*	0
	6	*30*	*4*	*34*	*3*	*38*	*1*	**167**	277
Tot. Obs.		21940	500	8085	446	2156	203	2348	**35678**

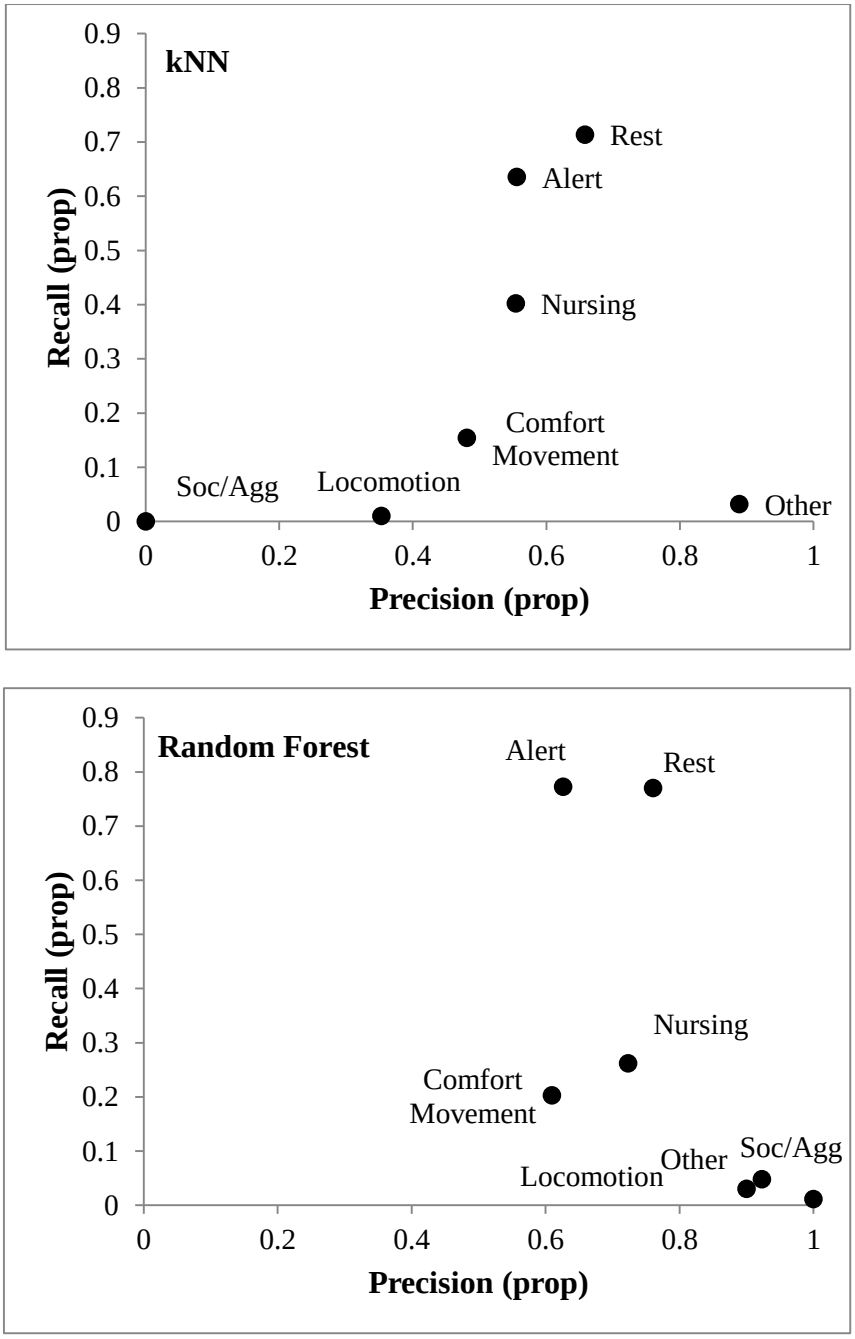


Figure 3.2: Plot of coarse-resolution behaviours (BCScoa) with respect to precision (proportion of positive behavioural classifications that were correct) and recall (proportion of new data pertaining to behaviours that were correctly classified as positive) for both the kNN and random forest models for head-mounted accelerometers for 2015. Behaviours include rest (0), social/aggression (1), alert (2), other (3), nursing/presenting (4), locomotion (5), and comfort movements (6) as defined in section **2.2**.

Of the feature variables calculated to summarize the acceleration data, components relating to static acceleration (those relating to body posture) were found to be the most important for classifying behaviours according to random forest models; stZ, stX, stY ranked as 1st, 2nd, and 5th and Pitch and Roll were ranked 3rd and 4th relative to the decreasing Gini index (**Figure 3.3**). Gini will approach zero as each of the branches contain a single behavioural category, therefore a greater decrease in mean Gini indicates that the feature variable in question is more important for splitting these branches and differentiating the behaviours within the random forest models. Summaries of these top five feature variables with respect to each of the behavioural states associated with BCScoa can be found in **Table 3.3**. The next most important variables were those related to dynamic body acceleration; both smoothed VeDBA and VeDBA ranked 6th and 14th (Appendix **Table A3.1**). Power spectrum densities in all acceleration dimensions were also found to be very important, ranking positions 7-12.

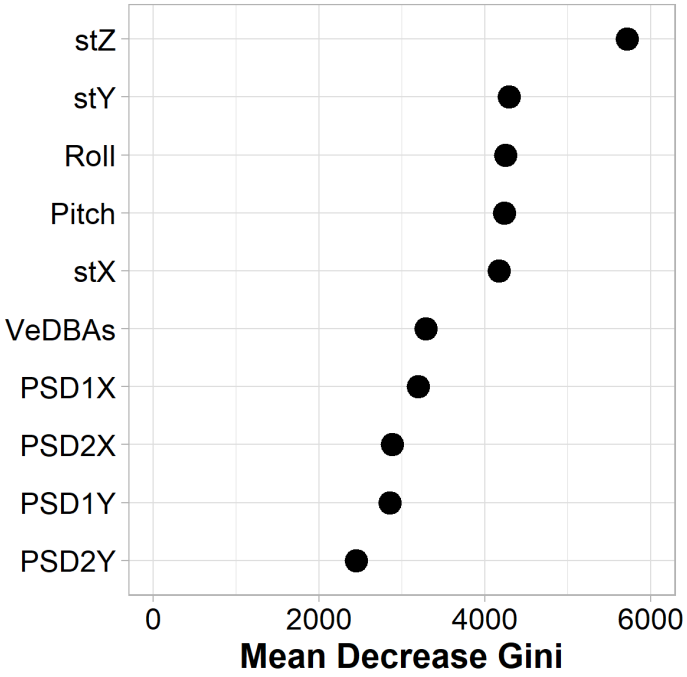


Figure 3.3: Top ten variable importance plot for random forest model classifying BCScoa representing decreasing mean Gini for each feature variable. Top feature variables included static acceleration and its derivatives, pitch and roll, as well as smoothed VeDBA and elements of power spectrum densities in the X and Y dimensions.

Table 3.3: Summary statistics for top 5 most important feature variables (superscript corresponds to definitions in **Table 2.3**) from the 7 behaviours classified from BCScoa using random forests on head-mounted acceleration data in 2015. These top 5 variables identified from highest decrease in mean Gini. Feature variables are summarized by median as well as 1st and 3rd quartile.

	stZ[3]			stY[2]			Roll[21]			Pitch[20]			stX[1]		
Beh.	Median	1st Q.	3rd Q.	Median	1st Q.	3rd Q.	Median	1st Q.	3rd Q.	Median	1st Q.	3rd Q.	Median	1st Q.	3rd Q.
0	0.721	0.045	0.836	-0.019	-0.280	0.340	-0.019	-0.284	0.347	-0.227	-0.422	0.153	-0.225	-0.410	0.153
1	0.868	0.800	0.909	-0.031	-0.186	0.105	-0.031	-0.188	0.105	-0.212	-0.348	-0.026	-0.210	-0.341	-0.026
2	0.818	0.722	0.879	-0.051	-0.213	0.108	-0.051	-0.215	0.108	-0.310	-0.434	-0.151	-0.305	-0.421	-0.150
3	0.795	0.697	0.849	-0.033	-0.188	0.100	-0.033	-0.189	0.101	-0.416	-0.593	-0.267	-0.404	-0.559	-0.264
4	0.208	-0.198	0.759	-0.194	-0.913	0.716	-0.195	-1.153	0.799	0.059	-0.120	0.238	0.059	-0.119	0.236
5	0.830	0.749	0.879	-0.021	-0.145	0.093	-0.021	-0.146	0.093	-0.288	-0.409	-0.139	-0.284	-0.398	-0.139
6	0.700	0.139	0.837	-0.024	-0.448	0.371	-0.024	-0.464	0.381	-0.145	-0.345	0.122	-0.144	-0.338	0.121

The effects of year and individual on the top feature variable, stZ, were modelled as a generalized linear mixed effects model with maternal post-partum mass included as a fixed effect. Overall, Presenting/Nursing and Locomotion were found to vary greatly between individuals in relation to stZ (**Figure 3.4**). The variance component due to individuals was 24.3 ± 19.9% for Presenting/Nursing and 12.9 ± 5.8% for Locomotion (**Table 3.4**). The remaining behaviours in BCScoa, however, showed less than 8% variance attributed to individual ID. No variance was explained by the effect of year across bootstrapped samples. However, top feature variables associated with each behaviour appeared to be repeatable across individuals, indicating varying degrees of stereotypy (**Table 3.4**). Alert and Other, largely upright behaviours, appear to be consistent for each seal with respect to stZ and its derivative Pitch, while Rest and Presenting/Nursing, where the head is most often tilting in a downward fashion, were repeatable with respect to stX (**Table 3.4**). Social/Aggression-type behaviours were found to be the least repeatable among the behaviours tested in BCScoa with respect to VeDBAs (*adj*-R = 0.037), following after Locomotion with respect to Freq1X (*adj*-R = 0.105). Comfort Movement was found to be highly and significantly repeatable between years as determined through a likelihood ratio test with respect to (*adj*-R = 0.603, D = 641, $p = 0.005$; **Table 3.4**).

Flippering pup (BCSmed behaviour '4'), integrated into behaviour '6' in BCScoa, was also tested as it was classified well in other BCS models and was found to be extremely repeatable and highly significant (*adj*-R = 0.925, D = 1070, $p < 0.001$; **Table 3.4**). This led to evidence that some females lay preferentially on one side of their body (indicated by Roll) during the Flippering pup behaviour (**Figure 3.5**). Four females were found to preferentially lay on their right side, where Roll was significantly less than 0 as determined through a one sample signed rank test (including females '0J': V = 148, $p < 0.001$; '74789': V = 1017, $p < 0.001$; '74904': V = 3598, $p < 0.001$; and '74962': V = 1207, $p < 0.001$; see **Figure 3.5**).

Likewise, five additional females were found to preferentially lay on their left size, where Roll was significantly greater than 0 as determined through a one sample signed rank test (including females ‘45447’: V = 145710, $p < 0.001$; ‘58038’: V = 46524, $p < 0.001$; ‘74920’: V = 475420, $p < 0.001$; ‘72146’: V = 1125800, $p < 0.001$; and ‘4H’: V = 84251, $p < 0.001$; see **Figure 3.5**).

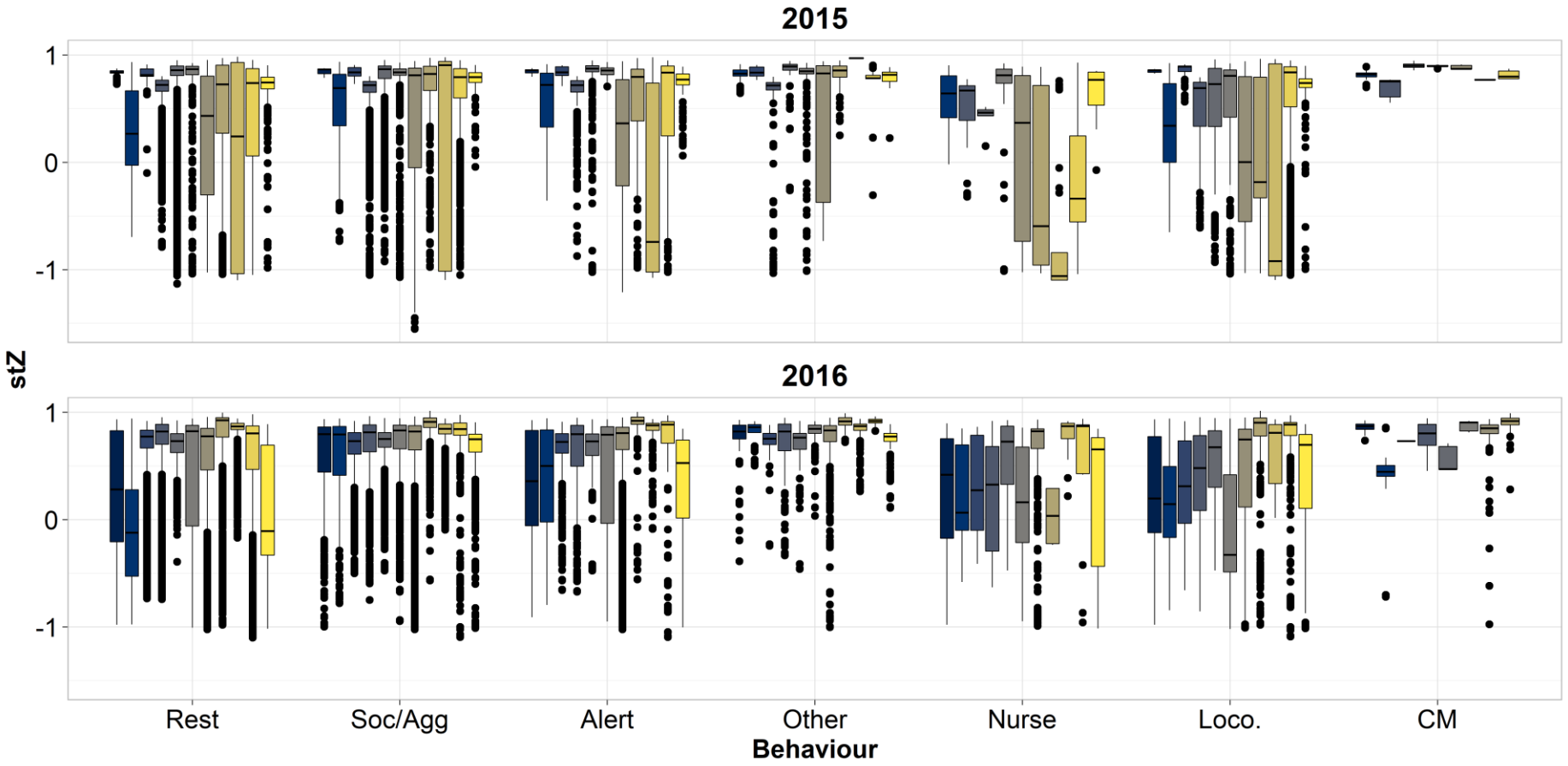


Figure 3.4: Boxplot of each behavioural group in BCScoa (0-Rest, 1-Soc/Agg, 2-Alert, 3-Other, 4-Nurse, 5-Loco., 6-CM) with respect to static acceleration in the Z-axis (stZ), the feature variable found to be most important in differentiating behaviour when modelled as a random forest model. This illustrates the high degree of individual variability (each shaded bar corresponds to a single study female recaptured between years) both within a single season (length of bars), as well as between the two years of study (top vs. bottom panels).

Table 3.4: Variance and repeatability estimates for BCScoa behaviours (0-6) with respect to repeat capture females (n = 11) over two years for the top feature variable, stZ. No variance was explained by year. Repeatability was estimated for each behaviour with respect to feature variables (and corresponding variable number, definitions in **Table 2.3**) thought to best describe each behaviour. Variance and adjusted repeatability (*adj*-R, adjusted for differences in maternal post-partum mass between years) was computed over 500 bootstrapped samples using the package 'rptR'. Comfort Movement (6) was found to be highly and significantly repeatable between years as determined through a likelihood ratio test (D = 641, p = 0.005, '*'). Flippering pup (BCSmed behaviour '4'), integrated into behaviour '6' in BCScoa, was also tested as it was classified well in other BCS models and was found to be extremely repeatable and highly significant (D = 1070, $p < 0.001$, '*').

	Behaviour	Variance	SE	Feature Var.	*adj*-R
(0)	Rest	0.042	0.019	stX^{1}	0.343
(1)	Social/Aggression	0.050	0.022	$VeDBAs^{12}$	0.037
(2)	Alert	0.077	0.038	stZ^{3}	0.338
(3)	Other	0.037	0.018	$Pitch^{20}$	0.563
(4)	Presenting/Nursing	0.243	0.199	stX^{1}	0.385
(5)	Locomotion	0.129	0.058	$Freq1X^{28}$	0.105
(6)	Comfort Movement	0.019	0.011	$Roll^{21}$	0.603*
	Flippering Pup	*0.058*	*0.029*	*$Roll^{21}$*	*0.925**

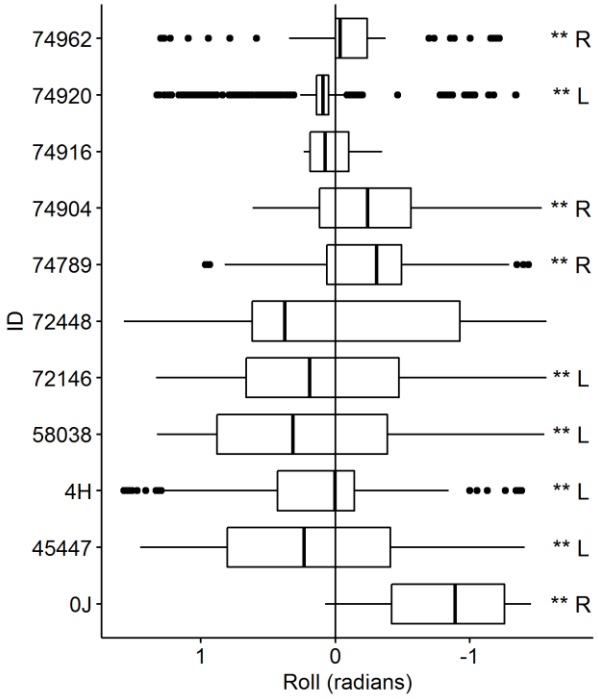


Figure 3.5: Boxplot of Roll as a derivative of static acceleration in the Y axis (stY) with respect to individual for repeat capture females associated with the flippering pup behaviour in BCSmed (see **2.5.1-A.4-FP**). Some females appear to show preference for being positioned on the left (values towards +1) or the right (values towards -1), potentially indicating individual lateralization in a female-pup interaction (flippering pup, FlipP). Significance was determined through a signed rank test and significant preferences denoted with either '**L' for an apparent left side preference or '**R' for an apparent right side preference.

3.4 – Discussion

Overall, behavioural classification using accelerometers of lactating grey seals produced mixed results. Generally, it was found that only a coarse level of resolution of behaviour could be identified, with BCScoa being the preferred classification model having classified the highest number of behaviours relative to its averaged F1 score. While this meant that fewer individual behavioural states were classified well, these behaviours represent upwards of 90% of a lactating female grey seal's activity budget, including Resting, Alert, and Presenting/Nursing in BCScoa as well as Flippering Pup in other models. Random forests consistently outperformed kNN models across all behaviours and accelerometer configurations. While issues in balancing sampling rate and length of deployments may have led to discrepancies in classification accuracy between years, many of the behaviours that were well classified were repeatable between years with respect to certain feature variables, though not necessarily significantly so. It was also found that individuals were not consistent in the mechanics of behaviour between years with respect to the training data, likely as a result of sampling bias and environmental context, and that individuals were highly variable in executing certain behaviours as noted with respect to top feature variable, stZ. In all, behaviours that were largely stationary, such as Resting and Presenting/Nursing, were better classified than those pertaining to directed movement, such as Locomotion. Accelerometers sampling at a higher frequency (50 Hz in 2015) than those sampled at a lower frequency (25 Hz in 2016) between years were better able to resolve Locomotory behaviours. Torso-mounted accelerometers generally performed better than head-mounted accelerometers on the same individuals. However, Locomotion was completely and unexpectedly undetected in torso-mounted accelerometers (25 Hz in 2016).

3.4.1 – Classification algorithms

Random forests have been used to classify behaviour in a wide range of taxa, including domestic sheep (*Ovis aries*; Alvarenga et al. 2016), Eurasian beavers (*Castor fiber*; Graf et al. 2015), brown hares (*Lepus europaeus*; Lush et al. 2015), puma (*Puma concolor*; Wang et al. 2015), griffon vultures (*Gyps fulvus*; Nathan et al. 2012), and other pinniped species (e.g. Ladds et al. 2016). Common to all these studies, behaviour could only be resolved to three or four behavioural states that are extremely disparate in their feature characteristics, as was the case in the current investigation. While random forests are much more computationally intensive to train as compared to simpler algorithms like kNN, they take much less time to classify new accelerometry data and are generally more robust given their two layers of randomness in classification (Cutler et al. 2007). On the other hand, kNN algorithms adopt a much more intuitive method of spatial classification, but training data is needed throughout the classification process and can eat up precious memory during run time. Classification through kNN can be very useful and easy to understand, especially when behavioural modes or states are very distinct and sufficiently different in feature space, as was the case when categorizing several behavioural states in Eurasian badgers (*Meles meles*; McClune et al. 2014). While Bidder et al. (2014) demonstrated its applicability across a number of test cases with different species, the kNN algorithm may be limited in its ability to classify behaviour without the use of thresholding to improve accuracy. Improved model fit may unfortunately be at the cost of a loss of information by being unable to classify behaviour segments that fail a set threshold. It was also noted that kNN algorithms may also be sensitive to minor discrepancies in device attachment or additions of noise to the data (Bidder et al. 2014).

While the random forest approach outperformed that of the kNN, it is worth noting that the Gini impurity index did not approach zero across our selected feature variables and behavioural categories in the preferred model. In the estimates of error across trees (**Appendix**

Figure A3.1), several of the behaviours with the poorest precision and recall also had the highest error rates. This reiterates the point that some of our behaviours of interest might be to variable in execution and duration to accurately classify given the study design, even with random forest models. In signal theory language, random signals, as might arise from animal behaviour, are very difficult to characterize (Cadzow and Van Landingham 1985). These signals are often contaminated with multiple spectral densities and frequencies that will vary in magnitude over time. Often these signals violate the assumptions of transforms, such as the fast Fourier transform used here, that may lead to inconsistent features, even when properly windowed through more advanced signal processing methods. The lack of consensus across trees with Gini not approaching zero may indicate that more feature variables may need to be included in order to more accurately classify these 'other' behaviours. It should also be noted that it may not be possible to accurately and consistently extract some of the behaviours of interest from acceleration data, even with the addition of more feature variables. Behavioural states also did not include blanking times in the accelerometry data. Removing periods of 'transitions' between behaviours may yield higher classification accuracy, but defining these periods remains subjective.

3.4.2 – Stationary behaviours during lactation

Overall, it was found that static acceleration and its subsequent components were considered the most important features for discriminating behaviour. This is not necessarily unexpected as seals often remain inactive for long periods of time. Often considered the most important behaviours during lactation, Resting (**2.5.1-A.1-REST**), Presenting/Nursing (**2.5.1-A.4-PN** and **PNAL**), and other largely stationary behaviours were easily identified in female grey seals on both head- and torso-mounted accelerometers (precision of 74% and 75%, respectively and recall of 96% and 62%, respectively). These behaviours involve extensive periods of little to no movement, with only periodic adjustments of body position lasting for

brief periods (e.g. **2.5.1-A.3-CM**). Resting and other static behaviours are often the most easily identifiable behaviours as found in a variety of taxa (e.g. Fossette et al. 2012; Wang et al. 2015; Portugal et al. 2016). It also may not be surprising that these behaviours were classified well and were ones in which static components were sufficiently different between behaviours in this species.

These Resting and Presenting/Nursing behaviours represent the key dynamism of energy conservation within the female during lactation to maximize the transfer of finite energy stores to the pup and represent anywhere from a combined 65-90% of a females activity budget in the wild (e.g. Kovacs 1987; Twiss et al. 2000, 2012a; Robinson et al. 2015). In the current study, these two behaviours represented approximately 40% of the testing data (see **Table 3.2b**). Grey seals, as previously mentioned, exhibit a capital breeding system whereby females do not return to sea to forage and supplement their energy stores (e.g. Mellish et al. 1999). Resting often seems to be viewed in ethology as the leftover period of a behavioural activity budget. In the case of breeding grey seals, this often overlooked behaviour is arguably one of the most important. Grey seals of both sexes must carefully budget time spent resting in order to maximize their internal resources (Sparling et al. 2006; Bishop et al. 2015b). For grey seal males, conserving time spent resting may increase tenure within a key breeding territory as they may spend several weeks on the colony without supplemental energy income (Bishop et al. 2017). While Resting can be variable in overall body positioning in lactating grey seals, Presenting/Nursing appeared stereotypical, as indicated by its moderate repeatability, with females alternating regularly between lying on the right or left side as indicated by the wide range of the static acceleration signal across years to maximize access to both teats (**Figure 3.4**); little to no lateralization was detected for Presenting/Nursing when investigated in detailed observational studies (S. Twiss and S. Ella, *pers.comm.*). Typically, maternal effort is quantified by the fat and protein content of milk, overall milk output, or enzyme activity levels

as an indication of fat mobilization and assimilation efficiency in the both female and pup, respectively (Iverson et al. 1993; Mellish et al. 1999a, 2000). These previous lactation studies often involved many repeated sampling events over the lactation period that likely cause disturbance to both the female and her pup. When these repeated sampling events are unavailable, the alternative is to infer energy usage through short-term fitness outcomes like mass-transfer efficiency determined from masses at the start and end of lactation (see equation **2-4**; e.g. (Pomeroy et al. 1999). Individual differences in physiology likely represent the bulk of variability in energy transfer, such as repeatable individual differences in milk fat content (Mellish et al. 1999a; b, 2000; Lang et al. 2009), internal enzyme activity and body tissue turnover (Mellish et al. 2000; Mellish and Iverson 2001), and hormone concentrations in grey seal pups and mothers (Robinson et al. 2019), though how these individual physiological differences arise is largely unknown (Lang et al. 2009). However, accelerometers may give a useful population-level estimate of maternal effort in Presenting/Nursing with minimal disturbance. While not a direct measure of energy transfer, this behaviour is likely a useful indication of extreme outliers of low mass-transfer efficiencies.

Interestingly, stationary pup interaction in the form of Flippering pup was also classified reliably in a variety of random forest models (**2.5.1-A.4-FP**; behaviour '4' in BCSmed and collapsed into '6' for BCScoa). While many other pup-directed behaviours can be identified through conventional behavioural observation, this was the only other maternal behaviour that was reliably classified in any of the BCS-levels investigated outside of Presenting/Nursing. Similar to Presenting/Nursing, females often engage in the flippering pup behaviour while lying on one side or the other, repeatedly stroking or scratching the pup. While this behaviour involves a similar body position to that of Presenting/Nursing or Resting, there is a slight average increase in the frequency in the X-axis associated with this behaviour, making it fairly stereotypical in feature space. As this behaviour is generally associated with

Presenting/Nursing events, this may be an important tool for further assessing patterns in mothering style. Interestingly though, some females appear to be selective in choosing to which side to lay on, likely using their opposite front flipper to stroke the pup, as indicated by significant tendencies towards positive (indicating left side preference) or negative (indicating right side preference) rotation in radians in Roll (**Figure 3.5**). There is increasing evidence towards preferential lateralization in mammals, both human and others (Hill et al. 2017; Karenina et al. 2017; Giljov et al. 2018). Lateralization, defined here as preferential orientation in the context of mothering dyads, has been noted to be associated with preferential swimming orientation cetaceans (Karenina et al. 2013, 2017; Hill et al. 2017), suckling behaviour (Schaafsma et al. 2009; Komárková and Bartošová 2013; Zoidis and Lomac-MacNair 2017), and a wide variety of other behavioural interactions in mammal species where preferences appear to align offspring with the right brain hemisphere associated with processing social cues (Schaafsma et al. 2009; Keerthipriya et al. 2015; Karenina et al. 2017, 2018; Giljov et al. 2018). The results presented here may indicate that female grey seals may exhibit a preference towards left handedness, whereby connecting to the right hemisphere by keeping the pup in the left eye (Hill et al. 2017). A preliminary analysis of video footage in grey seals has indicated that grey seals may also exhibit lateralization in a variety of non-nursing contexts (S. Twiss and S. Ella, *pers. comm.*). Results presented in this chapter provide further evidence for lateralization for non-nursing social interactions with a female and her pup, but should be explored further and across a wider range of behaviours.

3.4.3 - Vigilance

Grey seals have very few behavioural states that occupy more than a small percentage of their activity budget while on land. While behaviours like Resting, Presenting/Nursing, and pup interaction represent the core of a typical female's activity budget, behaviours like those associated with being in an Alert state may be an important indication of time and energy

trade-offs. Most often, vigilance behaviours (**2.5.1-A.2**), as well as other associated social (e.g. **2.5.1-A.5**) and aggressive interactions (e.g. **2.5.1-B**), were mostly mistaken for those of Resting behaviours. However, when collapsed into a broad categorization of Alert, such as in BCScoa behaviour '2', the behaviour could be extracted fairly accurately from the accelerometry data (precision 62% and recall 77%). Alert-type behaviours, even when the head is moving periodically to scan for threats, often involves many intermittent periods of relative stillness. What traditionally an ethologist might classify as several different combinations of vigilance and aggression behaviour over a period of 1 minute (**2.5.1-B.1** and **B.2**), an accelerometer might only characterize detectable movement that may be indistinguishable as separate states. In fact, Social/Aggression behaviours were most often mistakenly classified for alert behaviour in BCScoa (e.g. **Table 3.2b**). This indicates that these behaviours are best collapsed into a single broad alert category for behavioural classification using accelerometers, though at the loss of Social/Aggression information. Within the ethogram used in this study (section **2.5.1**), a mixture of states and brief events, such as pup-checking, were used to characterize behaviour. While accelerometers have been used for discrete event detections like head striking, as is seen in many of the aggressive interactions in the study females, this particular aspect of accelerometry is still in development and may not be yet applicable on a broader range of behaviours that vary in space and time (e.g. Ydesen et al. 2014). Higher sampling resolution may overcome this limitation, but at the cost of the length of deployment of accelerometers given current tag specifications.

Vigilance has been studied extensively in terrestrial species (Caro 1987; Loughry 1993; Burger and Gochfeld 1994; Arenz and Leger 1999). Often described in ungulates and other prey species, trade-offs in time allocation are mostly associated with balancing time foraging and acquiring energy (head-down) and looking out for potential sources of danger (head-up; Moreau et al. 2009; Alvarenga et al. 2016; Kölzsch et al. 2016). This variability in balancing

foraging time and vigilance drives much of what characterizes ecological aspects that define landscapes of fear and is often highly associated with a variety of density-dependent factors (Willems and Hill 2009; Gallagher et al. 2017; Stephens et al. 2019). In predatory contexts, spotted hyenas (*Crocuta crocuta*) are often seen to balance vigilance for prey sources with those of intraspecific aggression within social structures (Pangle and Holekamp 2010). Cheetahs (*Acinonyx jubatus*) likewise must strike a balance between remaining vigilant for conspecific threats to their cubs and the opportunity for prey items (Caro 1987). Grey seals, too, must balance the time that they spend vigilant watching out for threats to their young, such as harassment from other seals. While previous work has sought to investigate sociality and conflict reduction in grey seals (Ruddell et al. 2007; Bishop et al. 2017), the accelerometers used in this study were unable to accurately separate general alert behaviours from social interactions or threat displays. Most terrestrial studies evaluating vigilance have used collar-mounted accelerometers which aid in reliably extracting a variety of behaviours (Martiskainen et al. 2009; Signer et al. 2010). Given that our most important predictors of behaviour were static acceleration in all axes, the placement of an accelerometer in a location with a greater variety of postural dynamics, such as being glued on to the neck behind the head, may have yielded a more accurate picture of social and aggressive interactions.

3.4.4 - Locomotion

Identifying modes of locomotion is a popular aim in the accelerometry literature, from flight to running to swimming (Spivey and Bishop 2013; Maresh et al. 2015; Halsey 2016; Jeanniard-du-dot et al. 2016). This is likely because these modes of behaviour are easily identifiable in the spectral and time-frequency realms when consistent over long periods of time. Locomotion is often bounded by various biomechanical pressures that limit its interpretation (Schmidt-Nielsen 1972; King et al. 2004). Modes of locomotion, such as running or walking, can also often be explicitly separated by differences in the overall power

and spectral density (Wang et al. 2015). These modes of locomotion are so commonly separable that a lot of effort has gone into processing acceleration signals in real-time and have become a ubiquitous technology in fitness trackers for both humans and domestic animals (e.g. Godfrey et al. 2008; Robert et al. 2009; Mannini and Sabatini 2010). In marine mammals, differences in locomotion detected with tags mounted along the midline, often expressed as stroke frequency, are used as a reliable indicator of energetic expenditure at sea (Williams et al. 2004). Often, as in this study, these frequency and spectral density elements are extracted using a fast Fourier transform (Watanabe et al. 2005). This transform, derived from signal processing physics, assumes that the signal is stable in time and space in order to dissolve it into its spectral elements (Yost et al. 1983; Cadzow and Van Landingham 1985). Behaviours like swimming in marine mammals are often stable and can last over many minutes or hours. Windowing protocols often limit the amount of spectral leakage that may occur when these assumptions are violated. However, if a signal is too brief or inconsistent in execution, this transform is not likely to accurately detect changes in frequency and power; the signal may be missed entirely.

Even though elements of frequency-domain variables were within the top most important predictors of behavioural classification (see **Table A3.1**), Locomotion was only identifiable in head-mounted accelerometer deployments in the current study and was often confused with Alert or Resting behaviours (**Figure 3.2** and **Table 3.2**). It also had one of the lowest repeatability values in BCScoa (**Table 3.4**). In the case of grey seals on land, locomotion is brief as females tend to stay within a few body lengths of their pups, with only the rare long-distance trip to a pool of water as noted during *in situ* observations. In total, Locomotion-type behaviours only comprises a total of about 1% of a given female's overall activity budget (see **Table 2.2**), even across different seal breeding colonies (e.g. Robinson et al. 2015). Locomotion is clearly present within the accelerometry signal upon visual

inspection, with individual ‘steps’ visible, but generally was missed entirely by the classification algorithms used here as indicated by a high precision (mean 84.4%) and extremely low recall (mean 2.9%) when sampled at the highest rate in 2015 (**Table 3.2**). While there was a slight increase in recall between head- and torso-mounted accelerometers in 2016, the sampling rate was likely too low to accurately extract the relevant information for model detection and was completely undetected in torso-mounted accelerometers (**Table 3.1b** and **Table 3.2c**). It may be possible for accelerometers mounted on the torso, but sampling at a higher rate, to accurately detect Locomotion. In the ethogram used in this study to decode the video footage, three separate directed locomotion types were discriminated based on their apparent intensity including standard Locomotion (**2.5.1-A.3-LOCO**) as well as Fleeing and Chasing as a result of aggressive interactions (**2.5.1-B.2-CH** and **FL**). The BCSfine model attempted to separate these into two groups whereby fleeing and chasing might have higher frequency and magnitude elements but was unable to do so reliably (BCSfine behaviours ‘3’ and ‘7’; **Table 3.1d**).

Grey seal Locomotion may also be confounded with the effects of substrate and topographical context, alternating between vigilance and directed movement, as well as being able to locomote while still on its side (Tennett et al. 2018). While some individuals may differ, generally grey seals appear to limit the time spent locomoting (see **Table 2.2**), likely as a mechanism for conserving energy (Kovacs 1987). Female grey seals must prioritize maximizing energy stores upon arrival to a breeding colony to maintain themselves and nourish their pup during lactation while fasting; total body fat content is likely at its highest point in the year prior to lactation (Anderson and Fedak 1987). This means that female grey seals are not only prioritizing energy conservation to meet fitness goals, but also will incur a much higher cost of transport than other times of year where a female may be found on land, such as during the moult.

3.4.5 – Other behaviours

Despite occurring the least in the current dataset, the Other behaviour category corresponding to a drinking and snuffling behaviour was detected with moderate success but poorly classified (e.g. behaviour '5' of BSCfine; precision 78.9% and recall 7.4%, see **Table 3.1d**). This behaviour displayed unique head positioning to other behaviours whereby Pitch was highly repeatable within individuals (**Table 3.4**). Unsurprisingly, this was only apparently detectable in head-mounted accelerometers; the signal appears to be lost entirely in torso applications. As rarity of occurrence prevented accurate and consistent extraction of this behaviour, its application presented a unique opportunity to potentially evaluate the occurrence of drinking behaviour in grey seals. Future investigations may instead seek to extract this behaviour through event detection, rather than classification as presented here. One of the predictions of climate change in the coming decades may be an increase in the occurrence of drier and warmer periods overlapping with the breeding season on this colony (Jenkins et al. 2008). Grey seals have already been shown to prefer access to fresh water sources during lactation to drink and will travel variable distances in order to access them for thermoregulation (Redman et al. 2001; Twiss et al. 2002; Stewart et al. 2014). In truth, lactation costs account for a significant increase in the overall water flux of an individual with increasing metabolic output in mammals (e.g. Tedman and Green 1987; Lydersen et al. 1995; McLean and Speakman 1999). With some refinement in feature variable parameters in combination with an additional GPS or magnetometer, it may be possible to map the decision-making process in female grey seals in their use of freshwater pool systems and the associated behavioural changes with an increasing demand for supplemental water intake. In combination with more detailed thermal maps of temperature within a colony, one may be able to map the fine-scale extrinsic drivers and indicators of thermal stress that may result from a changing climate.

3.4.6 – Limitations of accelerometry and moving forward

The classification algorithms used here were only able to resolve behaviour to a coarse level of resolution, with context-dependent and interaction behaviours receiving the worst F1 scores (e.g. BCSmed behaviours '6' and '7' corresponding to male and female interactions, respectively, **Table 3.1c**). Several studies have also identified the confounding factors of classifying such contextual behaviours. One study on baboons found poor classification precision and recall when attempting to separate grooming behaviour when the individual was either the actor (grooming another) or the receiver (being groomed by another; Fehlmann et al. 2017). Another study in elephants showed that although differences in affective state could be discriminated, acceleration needed to be sampled at extremely high levels (1000 Hz) in order to elucidate minute differences in postural dynamics (Wilson et al. 2014). Given the inherent trade-offs in battery longevity, storage capacity, and sampling rate as well as best practice recommendations for tagging, it is unlikely that this type of highly sensitive measurement could yet be applicable in a wild setting. While not identified in the current study, a higher sampling rate may be able to highlight minute differences in postural dynamics that may improve in the identification of contextual interactions in grey seals. However, the resolution of behaviour that has been identified in the current study appears to be comparable to many other previous efforts to classify behaviour over extended periods in the field.

When examining interannual differences in behavioural mechanics for repeat capture females, it was found that individual ID and year included as random effects explained one fifth of the overall variance. While the exact reason for such a high amount of variance is unclear, study animals may impart an inherent confounding effect on behavioural classification from accelerometers, even when every effort is made to tag the same individuals. Several other studies have pointed out the potential confounding effects of environment in dictating the overall body position of an individual (Shepard et al. 2008; Halsey 2016). Static acceleration

was one of the most important predictors of behaviour in the favoured random forest model classifying 7 behavioural states in BCScoa (**Figure 3.3**). While female grey seals tended to return to similar locations on the colony between years, the topography of the island is highly variable and has already been shown to be an important consideration in the behaviour of this species (Pomeroy et al. 1994). Given this variability, it is more than likely that the inherent effects of topography confounded the consistency of classification precision and recall between individuals and between years. It is unclear how or if discrepancies in topography and their effect on body position and dynamic movement can be addressed or corrected for without the application of more sensors to model movement within quantified fine-scale topography, such as the addition of magnetometers, gyroscopes, or even GPS (e.g. Wilson et al. 2008). Comparisons to other breeding colonies with more homogenous topography may also serve as a useful measure of discrepancies in topography relative to behavioural classification. In addition, it is more than likely that higher precision and recall might be achieved if the behaviours were defined exclusively by their mechanics. This would, however, be at the risk of losing what little contextual information is contained in the behaviours that were attempted to be classified, which, arguably, is key to understanding the functions of such behaviours. Additional summary feature variables may also aid in resolving some of the less common behaviours but would vastly increase the dimensionality of the training data. Some work has looked into the development of hidden-Markov and state-space models as a way to classify changes in behavioural state and event detection (Grünewälder et al. 2012; Jonsen et al. 2013; Leos-Barajas et al. 2017). These methods principally rely on the selection of state-change probabilities in a similar manor to selecting priors in other Bayesian methods. While some have found improved accuracy, the selection of these priors may be subjective and require more development to detect more than two or three behaviours.

3.4.7 – Conclusions

In all, it was found that head-mounted accelerometers may identify a greater repertoire of behaviours using random forest models than with accelerometers mounted at the centre of gravity. Accelerometers placed on the centre of gravity appear to show promise in extracting a number of key behavioural states, though a higher sampling rate may be necessary to classify with greater precision and recall. Grey seals often remain inactive for long periods of time during lactation in order to conserve resources. Most of the movement is therefore limited to head movement or postural changes for nursing. While I achieved a coarse-level of behavioural resolution, it might be recommended to place accelerometers on the neck of breeding grey seals to access the greatest changes in position and postural dynamics. Likewise, while only a handful of behaviours were able to be extracted from torso-mounted accelerometers, these few states may be more important in quantifying differences in energetic expenditure. Improved accuracy could be achieved by attempting to classify fewer behaviours that are defined exclusively by their mechanics, but at potential loss of contextual and social information. It has also been shown that individuals may vary in the execution of behaviours in a wild context, supporting previous work that has flagged discrepancies within training data sets. Future work should consider this when training a classification algorithm using only a handful of animals as this may lead to poor detection in subsequent deployments.

Chapter 4:

Behavioural trade-offs in a capital breeder: Investigating coping styles and external pressures in lactating grey seals (*Halichoerus grypus*) using accelerometry and heart rate methods

Manuscripts from this Chapter:

SHUERT, C. R., P. P. POMEROY, AND S. D. TWISS (*in review*) Coping styles in capital breeders drive behavioural trade-offs in time allocation: Assessing fine-scale activity budgets in lactating grey seals (*Halichoerus grypus*) using accelerometry and heart rate methods. Behavioural Ecology and Sociobiology. [*Author Contributions*: CRS collected the data and performed the analyses with support from SDT and PPP. CRS wrote the paper with input from all co-authors]

TWISS, S. D., N. BRANNAN, C. R. SHUERT, A. BISHOP, P. P. POMEROY, AND S. MOSS (*in prep*) A robust externally mounted telemetry system for remotely recording heart rate and resting heart rate variability in free-ranging large wild mammals. Methods in Ecology and Evolution. [*Author Contributions*: All authors participated in field work, data collection, tag development, and validation. SDT performed analyses and wrote the paper with input from all co-authors]

4.0 – Abstract

Balancing time allocation among competing behaviours is an essential part of energy management for all animals. However, trade-offs in time allocation may vary according to the sex of the individual, their age, and even underlying physiology. During reproduction, higher energetic demands and constrained internal resources place greater demand on optimizing these trade-offs insofar that small adjustments in time-activity may lead to substantial effects on an individual's limited energy budget. This chapter investigated the potential underlying drivers of time-activity and trade-offs in a wild, capital breeding pinniped, the grey seal, during the lactation period using accelerometry and heart rate methods. For the first time, this thesis characterized full 24-hour activity budgets across the core duration of lactation as well as characterized how aspects of stress-coping styles, as determined through heart rate variability, influence time allocation. A distinct trade-off in time activity was found between times spent Resting and Alert (vigilance) as indicated by complementary models and trends. This trade-off varied with the pup's development, date, and maternal stress-coping style as indicated by a measure of heart rate variability, rMSSD. In contrast, time spent Presenting/Nursing did not vary across the duration of lactation given the parameters tested, outside of a mother's location on the colony. In all, this suggests that while mothers balance time spent conserving resources (Resting) against time expending energy (Alert), they are also influenced by the inherent physiological drivers of stress-coping styles. Incorporating the dynamics of these trade-offs on whole activity budgets as well as the modulating effect of stress-coping styles will help to better elucidate how individuals optimize energy usage trajectories over time and ultimately short-term fitness outcomes.

4.1 – Introduction

4.1.1 – Trade-offs in time as a finite resource

Changes in fine-scale behaviour are critical to examining trade-offs associated with time allocation. Activity budgets are a fixed and finite resource where time spent in one behaviour automatically creates a rebalancing of the remaining time spent in any other behavioural category (Altmann 1974). There are two main aspects to understanding time allocation: first, by quantifying the way an individual spends their time, as expressed through behaviour, and second, understanding the internal and external factors acting upon that individual, dictating the expression of behaviour. Sex-specific and age-specific trade-offs in behaviour have been examined extensively in the literature, not only for their relevance in the evolution of mating systems, but also to examine population trends over time as many of these differences dictate survival (Loison et al. 1999; Wolf et al. 2005; Byrnes et al. 2011; Hastings et al. 2011; Bishop et al. 2017). Long-term environmental factors are already known drivers of population-level behavioural trade-offs in a variety of taxa, and have been shown to be strong drivers of individuals balancing thermoregulatory needs with that of other behaviours (Liwanag et al. 2009; Turbill et al. 2011; Paterson et al. 2012; Heerah et al. 2013; Udyawer et al. 2017; Pagano et al. 2018). Environmental factors will likely become more important as the climate continues to change, especially with respect to high-latitude species for which homeostatic windows are much narrower (e.g. Rutishauser et al. 2010). Within a pinniped mating system, females will often exhibit differential investment of time, such as vigilance or aggression, and internal resources into offspring of different sexes (e.g. Anderson and Fedak 1987; Bowen et al. 1992; McMahon et al. 2016), which in turn may lead to differential survival for male and female pups after weaning (McMahon et al. 2000; Noren 2002; Hastings et al. 2009). The sex and age of a pup has already been shown to be a strong predictor of maternal behaviour in grey

seals (Kovacs and Lavigne 1986), but little work has been done to resolve how these intrinsic effects may be acting upon a full 24-hour activity budget.

More recently, consistent individual differences in behaviour, have been investigated as potential drivers of behavioural trade-offs (Dall et al. 2004; Sih et al. 2004). Consistent individual differences have been described in a variety of taxa, ranging from simple startle responses in the beadlet anemone (*Actinia equina*; Briffa and Greenaway 2011) and hermit crabs (*Pagurus bernhardus*; Briffa et al. 2008), to more complex differences in foraging strategies in other taxa (Patrick et al. 2014). Consistent individual differences thus represent a set of consistent behavioural responses across contexts, suggesting that individuals will tend to consistently exploit the same suites of trade-off strategies over time. The range of consistent differences are often determined through experimental set ups that may include mirrors (Dochtermann and Jenkins 2007), behavioural responses to unfamiliar stimuli (Twiss et al. 2012b), or response to handling events (Bremner-Harrison et al. 2004). This often results in an individual being placed on a spectrum of either stress-coping style or stress reactivity, depending on the context (Koolhaas et al. 1999). While these coping styles and stress reactivity measures can be an important indication of response to novel, controlled situations, only a few studies have examined their consequences in relation to whole activity budgets. While some studies have linked these trade-offs between stress-coping styles to fitness end points, such as body mass loss over lactation (Twiss et al. 2012a), it is unclear how they may drive overall trade-offs in time-activity in a free-ranging mammal.

Measurements of heart rate variability (HRV) have become a popular tool with which to evaluate individual differences in stress management (von Borell et al. 2007; Liu et al. 2014; Briefer et al. 2015). Various studies on captive laboratory animals have led to the definition of a spectrum of proactivity and reactivity that each characterize a suite of complimentary

feedback loops associated with the sympathetic and parasympathetic response pathways describing stress management strategies (Koolhaas et al. 2010). These pro-reactive coping styles are also characterized by a suite of different heart rate characteristics and behavioural traits (Koolhaas et al. 2011). Proactive individuals are typically characterized by low HRV as well as being behaviourally inflexible, routine-seeking, and generally more bold overall (Coppens et al. 2010). Reactive individuals on the other hand, tend to be characterized by high HRV and are generally much more flexible in their behaviour, responding more directly to changing inputs from the environment (Coppens et al. 2010). Since these stress-coping styles have already been shown to result in very different behavioural repertoires (Koolhaas et al. 1999), individual differences in HRV may lead to vastly different strategies in the overall activity budgets of female grey seals, especially in the face of differential extrinsic inputs. Given that grey seals have fixed energy reserves, small alterations in the behavioural repertoire of an individual may lead to large trade-offs on the path to meeting fitness goals, and ultimately offspring survival. As the climate continues to warm during the UK grey seal's breeding season (Jenkins et al. 2008), it is unclear whether individuals who are more stable or those who are more flexible will be able to manage an increasingly variable environment while still meeting ultimate fitness outcomes.

4.1.2 – Tracking fine-scale behaviour and time constraints in pinnipeds

Fine-scale behaviour over long periods of time has typically been unattainable through traditional methods. Observational studies typically involve long hours of work to decode extensive video footage of a few animals, or infer behaviour for an entire population through intensive scan sampling (Altmann 1974; Maniscalco et al. 2010; Witter et al. 2012). Many of these studies lack the means to observe behaviour overnight, outside of the core study area, or for individuals that are shy or cryptic in nature. Observer presence may also change behaviour, even when observed in a natural setting. Recent advances in biologging technology have

allowed for the collection of remote, fine-scale behaviour free from observer biases (e.g. Naito et al. 2010; Shaffer et al. 2014; Wang et al. 2015). Accelerometers overcome many of the limitations of observational studies, especially for the study of avian and marine mammal behaviour where individuals may remain inaccessible or out of site much of the time (Goldbogen et al. 2006, 2013; Sakamoto et al. 2009; Stothart et al. 2016). Recording continuously and at high resolution, tri-axial accelerometers can be used to remotely identify changes in body position, dynamic movement patterns, and even changes in affective state (Wilson et al. 2014). Prior to the development of accelerometers, the detail, scale, and length of behavioural observation was directly limited by observer effort, the pragmatics of observation, and other technical considerations. Accelerometers now seek to overcome the inherent limitations of traditional observation methods by allowing non-invasive methods to continuously monitor behaviour, even outside of an individual's observable period.

Pinnipeds represent an interesting case study for the use of accelerometry to determine fine-scale behavioural trade-offs. Telemetered pinniped research has typically focused on their at-sea behaviour, including dive profiles (Andrews and Enstipp 2016), foraging (Viviant et al. 2010; Skinner et al. 2014; Jeanniard-du-dot et al. 2016), and head-striking rates as a means of determining prey consumption (Skinner et al. 2010; Ydesen et al. 2014), as well as linking behaviour to fine-scale energetics (Williams et al. 2004). Very little research, however, has focused on the consequences of fine-scale behaviour during the terrestrial phase of pinniped life history, reproduction and nursing, using animal-borne telemetry devices. At breeding colonies located around the UK in the autumn, female grey seals haul-out to give birth to and nurse their pups over an average lactation period of about 18 days, where females may lose up to 60% of their initial body mass (Lydersen and Kovacs 1999; Mellish et al. 1999a, 2000). The conservation of energy resources is therefore paramount in maximizing this mass-transfer efficiency between a female and her pup, as monitored by a females activity budget over

lactation (Mellish et al. 1999a). Females must find a fine balance between conserving energy (Resting), transferring energy to her pup (Presenting/Nursing), and remaining vigilant (Alert) to protect both her pup and energetic resources in order to maximize fitness outcomes without compromising condition prior to the start of the annual moult or subsequent breeding attempts (e.g. Pomeroy et al. 1999). Balancing these three behavioural states likely represent a trade-off in time activity where too much time spent expending energy by remaining alert may have a detrimental effect on conserving energy (Resting) and transferring energy to the pup (Presenting/Nursing). Vigilance (Alert) has already been studied extensively in terrestrial mammals in the context of trade-offs in time-activity (Caro 1987; Arenz and Leger 1999; Beauchamp 2007; Pangle and Holekamp 2010). Consistent individual differences in time spent alert has already been suggested as an important indicator of stress management and coping styles in grey seals, and may have much larger implications in their fitness outcomes (Twiss and Franklin 2010; Twiss et al. 2012b).

4.1.3 – Study goals

The aim of this study was to outline potential predictors of time spent in certain target behavioural categories in lactating grey seals where trade-offs in time-activity are likely to exist. Behavioural activity budgets were generated from head-mounted accelerometers deployed over two successive breeding seasons. Behaviours of interest included time spent Resting, typically representing 50-70% of a female's activity budget (**Table 2.2**; Kovacs and Lavigne 1986; Twiss et al. 2000; Fraser et al. 2019), as well as time spent Alert and time spent Presenting/Nursing. While these behaviours could not be modelled together, these three behaviours represent the core of a female's activity budget and likely contain the most information regarding trade-offs in behaviour. Predictors of time spent in a given behaviour were investigated separately via binomial generalized linear mixed effects models (GLMMs) including factors intrinsic and extrinsic to each female-pup pair. A variety of heart rate

variability metrics as a measure of stress-coping styles (e.g. Marchant-Forde et al. 2004) were also investigated in predicting time spent in each of these behaviours. Based on previous investigations, I expect that females invest more time Alert and Presenting/Nursing male pups than female pups (Anderson and Fedak 1987), that individuals may be Resting more overnight (Culloch et al. 2016; Fraser et al. 2019), and that these behaviours all fluctuate as the pups age (Bowen et al. 1992). However, no study to date has been able to apply these questions of trade-offs in time to whole activity budgets, nor the role of individual differences in stress management in modulating these trade-offs.

4.2 – Methods

4.2.1 – Accelerometer-derived activity budgets

Over two consecutive breeding seasons (2015 and 2016), 38 female grey seals were equipped with small head-mounted data-logging tri-axial accelerometers (AXY-Depth, Technosmart Europe, Italy) for the core of lactation while on land (capture and tagging procedure outlined in **2.2** and **2.3**). Only those individuals that were sampled for a minimum duration of 6 days to capture the core of lactation were included in this study. Accelerometers were sampled at 50 and 25 Hz for the 2015 (n_{ind} = 18) and 2016 (n_{ind} = 20) autumn breeding seasons, respectively (n_{ind} = 7 recaptures). Accelerometer data were transformed and summarized according to 33 feature variables as outlined in **2.6** (**Table 2.3**), time-matched to video footage of behaviours as described in **3.2.3b**, and used to train a random forest model (n_{trees} = 500) with 7 possible behavioural states ('BCScoa', see section **3.3**, **Table 2.2**). This final random forest model was used create activity budgets over the length of the deployment of each accelerometer. For the purposes of this analysis, focus was placed on three behaviours of interest where trade-offs in time allocation were likely to exist including Resting (see **Table 4.1**: BCScoa behaviour '0'), Alert (behaviour '2'), and Presenting/Nursing (behaviour '4'). It is important to note that Presenting/Nursing includes periods where the female is lying

laterally, but may not always have a pup actively suckling. This behaviour should therefore be considered the total maternal effort in nursing. The proportion of time spent in each of these behaviours were summarized by consecutive day and night period, split by the timing of civil twilight in order to match previous efforts to study activity (e.g. Murie and Lavigne 1991; Harris et al. 2001; Culloch et al. 2016); day light hours included twilight at both ends of what constituted a day.

Table 4.1: Description of behaviour states for assessing time trade-offs in lactating female grey seals as well as the mean proportion (± sd) of time spent in each behavioural state across deployments as classified through accelerometry (behaviour definitions condensed from **2.5.1** and BCScoa).

BCScoa	Behaviour	Description	Prop.
0	Resting	Seal is motionless, head on ground	0.607 ± 0.11
2	Alert	Seal is stationary, head is up and moving, may look at pup	0.303 ± 0.11
4	Presenting /Nursing	Female is rolled laterally so that nipples are presented to pup; pup may be nursing	0.051 ± 0.05

4.2.2 – Factors influencing behaviour

Predictors of time spent in three key behavioural states, Resting, Alert, and Presenting/Nursing, were modelled separately using binomial GLMMs with a logit link function (Quinn and Keough 2002; Bolker et al. 2009; Zuur et al. 2009). Predictors of time spent in each behaviour were included as fixed-effects that were either intrinsic to each female-pup pair, extrinsic variables acting upon each female-pup pair, or elements of HRV relating to stress management unique to each female. As target female arrival on the colony was closely monitored, the parturition date for our study females in each breeding season was known or closely estimated to within a day. Using this date as well as mass at first capture, maternal post-partum body mass was estimated (see **2.7**). The ratio of maternal post-partum mass to maternal length was used as a proxy for female condition (here referred to as 'size') at the start of lactation and was included as a fixed effect. Other intrinsic factors such as the stage of the

pup on a given day (as outlined in **2.2**) and pup sex (modelled as 0/1, female is positive condition) were also included as fixed effects. Phase of lactation (Lphase) was also included as a potential predictor and was split into three inclusive bins (*early* – 1-6 days, *mid* – 7-10 days, *late* – 11+ days post-partum; Woldstad and Jenssen 1999). For extrinsic factors, weather variables were collected continuously across each breeding season, though methods differed between years (outlined in **2.8**). For the purposes of this analysis, daily maximum temperature (DMT), mean wind speed (Mwind), and daily total precipitation (Precip) were included as well as a female's location within the colony blocked into 7 distinct geographic regions on the island (**Figure 4.1**).

Over the course of accelerometer deployments, study females were also equipped with heart rate monitoring activity belts (**2.3**). These heart rate tags actively transmit data rather than logging, therefore recordings of heart rate were only available in real-time to a single, portable base station within a relatively short range (50-200m) within the colony. While this means that the heart rate data were not sampled continuously like the accelerometers, a sampling of heart rate over resting periods for most individuals allowed for the extraction of key summary variables describing heart rate and HRV, used as a proxy for stress-coping style across the pro-reactive behavioural spectrum as well as daily energy expenditure (Koolhaas et al. 1999, 2010; Coppens et al. 2010; Halsey et al. 2019). Key heart rate parameters included as fixed effects were the root mean square of successive differences in inter-beat intervals (rMSSD) and mean heart rate (mean-f_H), indicating stress-coping style as well as daily energy expenditure, respectively (Mohr et al. 2002; Marchant-Forde et al. 2004; Halsey et al. 2019). While elements in the time-domain indicate the totality of response associated with coping style, frequency-domain elements may be a better indicator of the separate influences of the sympathetic and parasympathetic responses to natural stressors. Frequency-domain indices of HRV included the mean of the parasympathetic nervous system indicator (mean.PNSI),

calculated as the ratio of high frequency components to total signal power (Marchant-Forde et al. 2004), and the median ratio of low frequency (0.03-0.15 Hz) to high frequency (0.15-2 Hz) components (median.LFHF; (Mohr et al. 2002). In addition, elements describing short-term HRV in the frequency-domain included the ratio of the median value for standard deviation of two dimensions of heart rate data calculated from the resulting ellipses within a Poincaré plot (SD2:SD1; (Hautala et al. 2010). These heart rate metrics were used to determine which metric may best characterize these differences, as well as determine whether differences in heart rate or HRV had an effect on time activity.

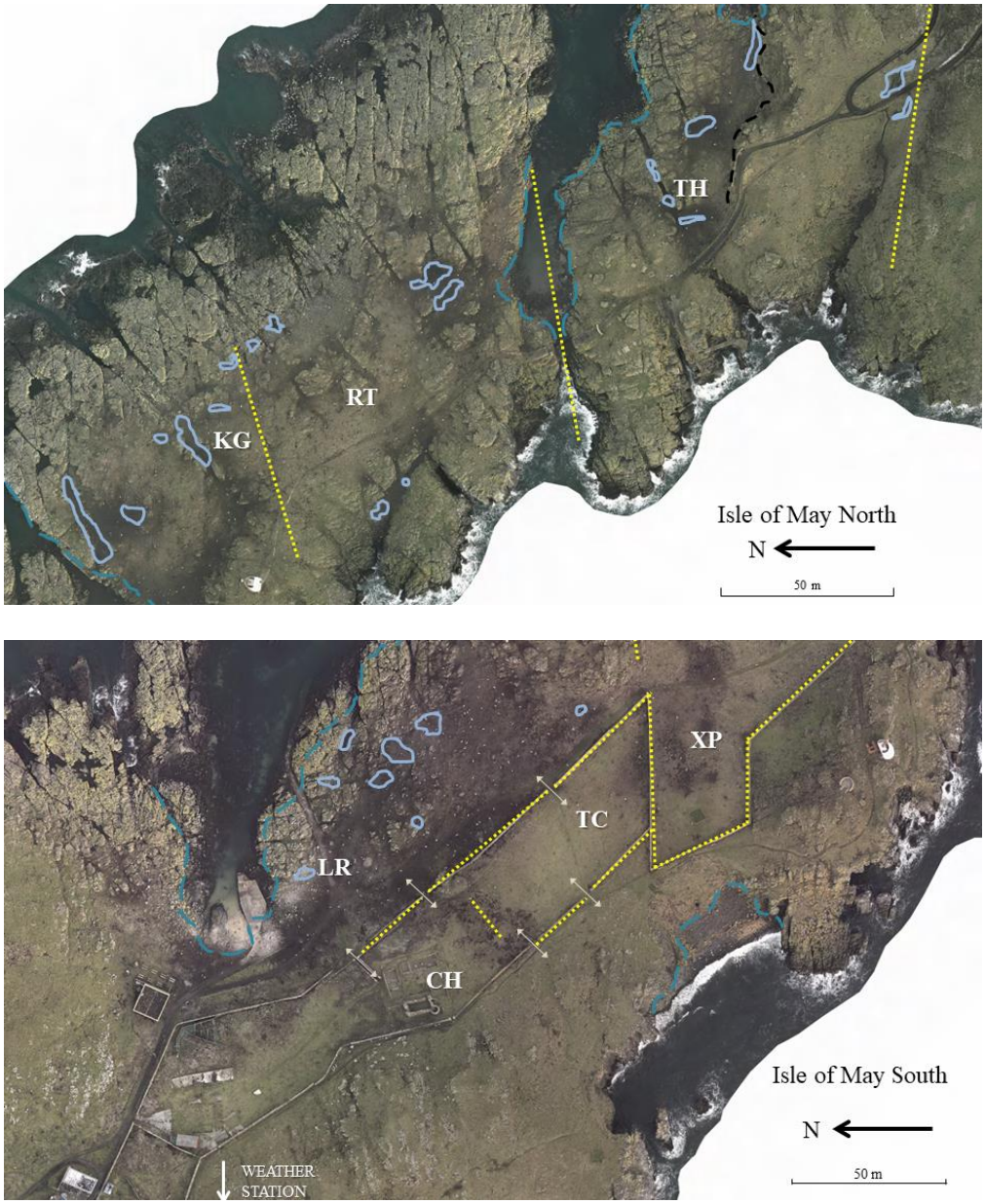


Figure 4.1: Map of colony sub-locations within the Isle of May. Locations include Karen's Gully (KG), Rona Top (RT), Tarbet Hole (TH) at the North end of the island and Loan Road (LR), Cross Park (XP), Tennis Courts (TC), and the Chapel (CH) to the South. Light blue areas represent relatively stable bodies of water located around the colony. Dark blue lines show approximate areas along the colony that are tidally influenced and are periodically flooded. Light arrows in the Southern end of the island indicate passages through dry stone walls between colony locations (Photos: SMRU aerial survey composite).

4.2.3 – Model parameterization

As this was the first opportunity to access the full suite of night behaviour, time spent in each behaviour was parameterized for each consecutive day and night (dn) for the length of each female deployment. Intrinsic, extrinsic, and HRV factor models were designed *a priori*

and are listed in **Table 4.2**. Pup sex was included in all models as it has already been shown to influence how a female partitions her time; females may invest more energy and time into male pups than female pups (Anderson and Fedak 1987; Kovacs 1987). As a result, the null model presented here is reduced to include only pup sex and dn to capture what is already known to influence activity budgets in female grey seals.

Table 4.2: Predictors for generalized linear mixed effects models (GLMM) including (A) intrinsic variables, (B) extrinsic variables, and (C) measures of HRV used to assess time spent Resting, Presenting/Nursing, and Alert with female ID included as a random variable. Intrinsic predictors include the phase of lactation (Lphase), date (modelled as a second-order polynomial), and pup stage. Maternal size was calculated as the ratio of mass to length of the female. Extrinsic predictors include daily maximum temperature (DMT), mean wind speed (Mwind), total daily precipitation (Precip), date, and colony location. Heart rate variability measures are described in the Methods. All models included pup sex and day/night (dn), including the null.

	No.	Binomial GLMM Predictors
(A) Intrinsic Models	1	Lphase + Date* + PupStage + PupSex + size + dn
	2	Date* + PupStage + PupSex + size + dn
	3	Lphase + PupStage + PupSex + size + dn
	4	Date* + PupSex + size + dn
	5	Lphase + PupSex + size + dn
	6	PupStage + PupSex + size + dn
	7	PupStage + PupSex + dn
	8	PupSex + dn (null)
(B) Extrinsic Models	1	DMT + Mwind + Precip + Date* + ColLoc + PupSex + dn
	2	DMT + Precip + Date* + ColLoc + PupSex + dn
	3	DMT + Mwind + Date* + ColLoc + PupSex + dn
	4	DMT + ColLoc + PupSex + dn
	5	Precip + ColLoc + PupSex + dn
	6	Mwind + ColLoc + PupSex + dn
	7	ColLoc + PupSex +dn
	8	DMT + PupSex + dn
	9	Mwind + PupSex + dn
	10	Precip + PupSex + dn
	11	PupSex + dn (null)
(C) Heart Rate Var. Models	1	rMSSD + PupSex + dn
	2	mean-f_H + PupSex + dn
	3	mean.PNSI + PupSex + dn
	4	median.LFHF + PupSex + dn
	5	SD1:SD2 + PupSex +dn
	6	PupSex + dn (null)

All continuous variables were scaled and centred to meet model assumptions
* Date modelled as a second order polynomial

A preliminary analysis found that the behavioural data were overdispersed. Female identity was included as a random effect in all models to not only account for overdispersion, but also to account for individual variability in behaviour (Bolker et al. 2009). Behavioural data were modelled as the proportion of time spent in the behaviour of interest, weighted appropriately for the total number of seconds in each day and night period from the accelerometer data, as each day of study loses about 5 minutes of daylight. All models were ranked based on Akaike Information Criterion, corrected for small sample size (AICc; (Anderson et al. 1998) and model deviance. Models were initially designed in three different groups including intrinsic factors only, extrinsic factors only, and HRV indices separately. This was done in order to tease apart the potential trade-offs associated with separate influences of intrinsic, indices HRV and stress response, and extrinsic drivers of behaviour, without masking any important factors that may be lost within model selection methods.

Top models were selected using AICc model selection methods (Burnham and Anderson 2002). Those factors included within 2 delta AICc were considered to have extensive support in modelling, while those with ≤ 5 delta AICc were considered to have minor support. Models with more than 7 delta AICc were not considered in model selection methods (Burnham and Anderson 2002). Model covariates with extensive or minor support from each of the three model groups (intrinsic, extrinsic, and HRV) were combined into a final model list and competed against the null for each of the three behavioural states to identify ultimate trade-offs. Models were described by their deviance and Akaike weight, with evidence ratios, calculated as the ratio of model weight to the null model containing only pup sex and dn (Burnham and Anderson 2002; Burnham et al. 2011). All models were built using the 'glmmTMB' package, ranked using the 'bbmle' package, and covariates model averaged with the 'MuMIn' package in R (Bolker and Team 2017; Magnusson et al. 2017). Any post-hoc

differences in model variables were assessed through Wilcoxon rank sum tests (Quinn and Keough 2002).

4.2.4 – Interannual consistency in trade-offs

The consistency in trade-offs in time-activity between consecutive years was also investigated. Top model covariates from the previous analysis (those variables with at least minor support) were assessed for repeatability (*adj*-R) for females that were recaptured between the 2015 and 2016 seasons (n_{ind} = 7). Repeatability of time spent in each behaviour (Resting, Presenting/Nursing, and Alert) and within-female variance between years was estimated through a bootstrap of 500 samples using the 'rptR' package and significance was assessed through likelihood ratio tests (LRT; Stoffel et al. 2017).

4.3 – Results

4.3.1 – Resting

Minor support (≤ 5 delta AICc) was found for the inclusion of pup stage as a predictor of time spent Resting in addition to the mandatory inclusion of pup sex and the effects of day and night (dn) as predictors. Females spent significantly less time resting with female pups (Wilcoxon rank sum test; W = 57330, p = 0.006) and rested significantly more at night (W = 28550, $p < 0.001$). Females rested significantly longer on average when pups were in stage II (W = 15146, $p < 0.001$) and significantly less when the pups were stage IV (W = 35062, p = 0.006; **Figure 4.2**). A *post-hoc* analysis of intrinsic and extrinsic models found that female size as well as all other weather variables were likely 'pretending variables,' as they failed to improve deviance compared to those models that did not include each of these factors (see **Appendix 4.1**; Anderson 2008). As a result, models including these factors were removed from model competition for time spent Resting (see **Appendix Table A4.1**). Little to no support was found for more complicated models including colony location or other intrinsic variables, with the exception of pup stage and date. Model weights were recomputed after the

removal of pretending and unsupported variables. The HRV metric rMSSD found extensive support (≤ 2 delta AICc, **Appendix Table A4.1c**) when compared to the other HRV metrics, yielding an evidence ratio of 4.015 above the next HRV model and 52% of the model weight. Females with higher rMSSD tended to rest for longer than those with lower HRV, with an apparent inflection point around 55 (**Figure 4.3**). Resting also appears to be highest at the start and end of season, as indicated by positive relationship for the density-dependent effect of date. The combined evidence ratio for the inclusion of rMSSD along with pup stage and date was 26.77 above the null model (pup sex and dn only; **Table 4.3A**). Final models, rankings, and recomputed Akaike weights are included in **Table 4.3A**. Beta estimates were model averaged across these final top models and reported in **Table 4.4A**.

Table 4.3: Final models for predicting time spent Resting (A), Presenting/Nursing (B), and Alert (C) in lactation female grey seals with extensive and minor support, following the removal of several pretending variables as determined by a *post-hoc* analysis. Models were ranked based on Akaike Information Criterion, corrected for small sample size (AICc). Time spent Resting and Alert were best described by pup stage, date, and the heart rate variability metric rMSSD. Time spent Presenting/Nursing was best described by location on the colony. Pup sex and differences in day and night activity (dn) were included in every model.

(A) Resting

Model Formula	*k*	Delta AICc	Weight	Deviance
Date* + PupStage + rMSSD + PupSex + dn	8	0.0	0.417	1367.099
Date* + rMSSD + PupSex + dn	7	0.9	0.264	1370.067
PupStage + rMSSD + PupSex + dn	6	2.0	0.151	1373.224
rMSSD + PupSex + dn	5	2.3	0.133	1375.521
PupSex + dn	4	4.9	0.036	1380.179

(B) Presenting/Nursing

Model Formula	*k*	Delta AICc	Weight	Deviance
ColLoc + PupSex + dn	10	0.0	0.89	649.981
PupSex + dn	4	4.1	0.11	666.336

(C) Alert

Model Formula	*k*	Delta AICc	Weight	Deviance
Date* + rMSSD + PupSex + dn	7	0.0	0.455	1304.548
Date* + PupStage + rMSSD + PupSex + dn	8	0.3	0.390	1302.803
rMSSD + PupSex + dn	5	3.3	0.089	1311.888
PupStage + rMSSD + PupSex + dn	6	4.3	0.053	1310.908
PupSex + dn	4	7.1	0.013	1317.758

*date modelled as a second order polynomial

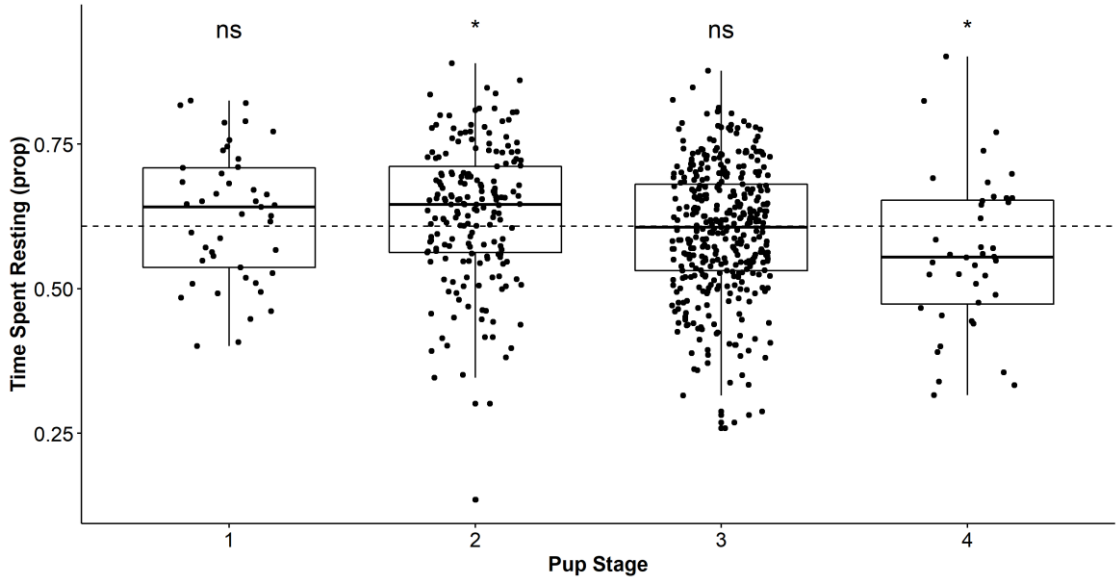


Figure 4.2: Boxplot of time spent resting as a function of pup stage in grey seals. The dashed horizontal line represent the overall mean time spent Resting. Females spent significantly longer Resting when pups were stage 2 than average, and significantly less in stage 4, as indicated by the (*) above each boxplot. Stage 1 and 3 were not significantly different than average (ns). Significance was found by a Wilcox rank sum from the mean time Resting across lactation.

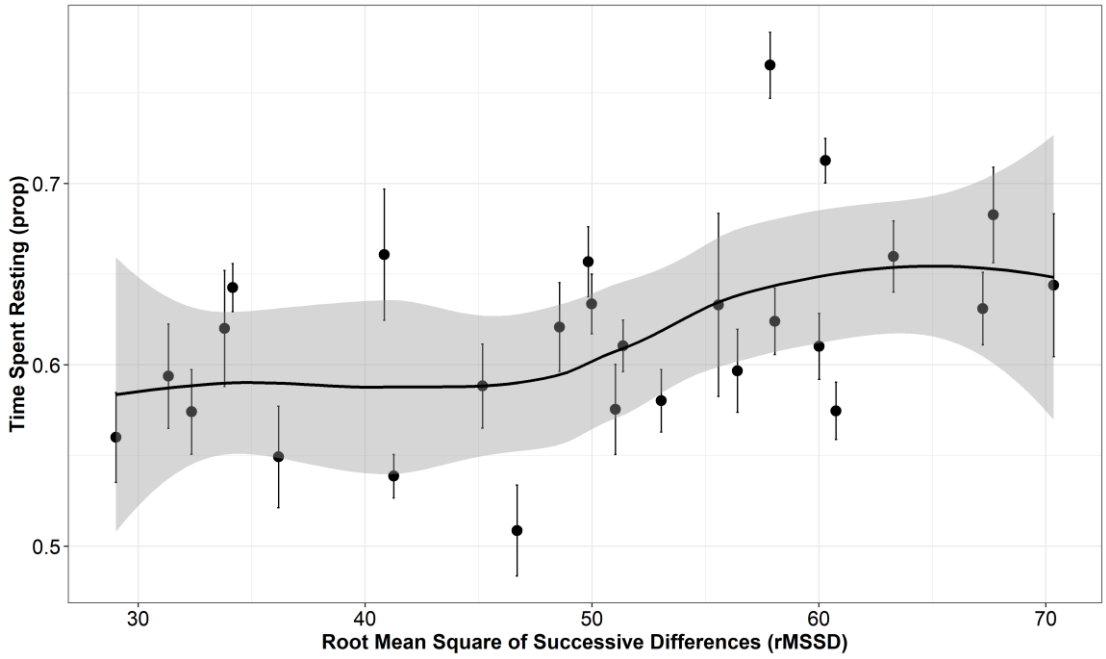


Figure 4.3: Scatter plot of time spent Resting as a function of the root Mean Square of Successive Differences (rMSSD) describing heart rate variability in female grey seals. Point values represent the mean time spent resting by each female, with standard error represented by the black lines. The black line represents a loess-smoothed trend line with 95% confidence intervals around the line in grey.

Table 4.4: Model averaged parameter estimates of top binomial GLMMs describing time spent Resting (A), Presenting/Nursing (B), and Alert (C) in female lactating grey seals. Top model predictors included date (modelled as a 2nd order polynomial), pup stage and rMSSD with pup sex (positive condition is female) and day vs. night (dn, positive condition is night) included in all models for Resting and Alert. Time spent Presenting/Nursing was best described by colony location (ColLoc) only (see **Figure 4.1** for definitions, against KG). In order to meet model assumptions, rMSSD was scaled and centred.

				95% Confidence	
	Parameter	Estimate	Std. Error	Upper	Lower
(A) Resting	(Intercept)	0.502	0.195	0.884	0.119
	Date – poly 1	1.847	1.194	4.187	-0.493
	Date – poly 2	1.845	1.140	4.079	-0.389
	PupStage	-0.099	0.060	0.018	-0.216
	rMSSD*	0.083	0.040	0.161	-0.004
	PupSex (F)	-0.144	0.083	0.018	-0.306
	dn (night)	0.373	0.078	0.525	0.220
(B) Presenting/Nursing	(Intercept)	-2.121	0.429	-1.280	-2.961
	ColLoc: LR	-0.337	0.436	0.517	-1.191
	ColLoc: RT	-1.265	0.393	-0.494	-2.035
	ColLoc: TC	-1.305	0.431	-0.462	-2.149
	ColLoc: TH	-0.725	0.301	-0.135	-1.315
	ColLoc: XP	-1.249	0.328	-0.606	-1.891
	ColLoc: CH	-1.144	0.655	0.139	-2.427
	PupSex (F)	-0.164	0.196	0.220	-0.548
	dn (night)	0.087	0.162	0.404	-0.230
(C) Alert	(Intercept)	-0.818	0.177	-0.471	-1.164
	Date – poly 1	-2.464	1.256	-0.002	-4.925
	Date – poly 2	-2.176	1.223	0.221	-4.573
	PupStage	0.082	0.065	0.209	-0.045
	rMSSD*	-0.103	0.042	-0.020	-0.185
	PupSex (F)	0.189	0.089	0.363	0.014
	dn (night)	-0.427	0.083	-0.264	-0.589

* scaled and centred

4.3.2 – Presenting/Nursing

Extensive support was found for an effect of colony location time spent Presenting/Nursing in addition to the predictors of pup sex and dn. No support was found for any single HRV metric in predicting time spent Presenting/Nursing as all models were equal in predictive power to that of the null (≤ 2 delta AICc; see **Appendix Table A4.2c**). There was no overall significant difference between time spent Presenting/Nursing male and female pups (Wilcoxon rank sum test; W= 66064, p = 0.157), but mothers tended to spend significantly

more time Presenting/Nursing at night (W= 58421, $p = 0.0273$). Mothers also varied significantly in Presenting/Nursing time across the seven colony locations (Kruskal-Wallace rank sum test: KG $H(2) = 39.83$, $p < 0.001$; LR $H(2) = 15.43$, $p < 0.001$; RT $H(2) = 8.63$, $p = 0.003$; TC $H(2) = 7.86$, $p = 0.005$; TH $H(2) = 14.41$, $p < 0.001$; XP $H(2) = 18.47$, $p < 0.001$; CH $H(2) = 2.20$, $p = 0.13$; **Figure 4.4**). Females at Karen's Gully (KG) and in the Loan Road (LR) had the highest average time Presenting/Nursing. These areas are located near large and permanent sources of water, while those in Cross Park (XP) with no access to water had the lowest average time Presenting/Nursing ($p < 0.001$; **Figure 4.1**). Colony location on its own led to an evidence ratio of 8.091 over the null model (pup sex and dn, only) after the *post-hoc* removal of weather variables as pretending variables (**Table 4.3B**, **Appendix Table A4.2a** and **b**). Beta parameter estimates were model averaged for top model parameters of time spent Nursing and can be found in **Table 4.4B**.

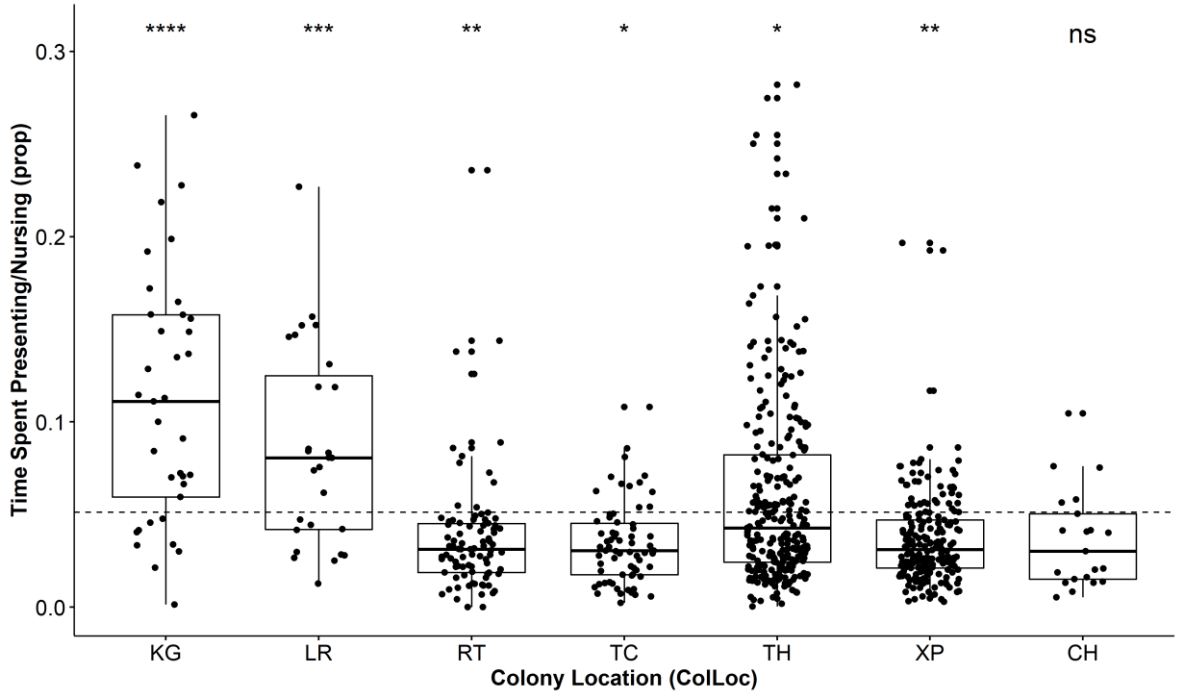


Figure 4.4: Boxplot of time spent Presenting/Nursing within each colony location (see **Figure 4.1** for definitions). The dashed horizontal line represent the overall mean time spent Presenting/Nursing. Females at KG and LR spent the longest average time Presenting/Nursing, while those in XP had the least. Significance of deviation from mean (indicated by * or ns across the top, mean indicated by dashed line) was determined through a Kruskal-Wallace rank sum test for each location.

4.3.3 – Alert

Again, extensive support was found for rMSSD in predicting time spent Alert against other HRV variables, with an evidence ratio of 8.048 above the next best model (**Appendix Table A4.3c**). Females with a lower HRV (as indicated by a lower rMSSD value) spent a greater portion of their time Alert than those with a more variable heart rate (**Figure 4.5**), with an apparent inflection point again at around 55. Females spent significantly more time alert with female pups than male pups (Wilcoxon rank sum test; W = 43820, $p = 0.002$). Mothers also spent a significantly shorter time Alert at night (W = 74664, $p < 0.001$). As with Resting, extensive support (≤ 2 delta AICc) was found for pup stage and date. Mothers varied significantly across the four stages of pup development, spending a significantly shorter time Alert during stage II of pup development (W = 49104, $p = 0.001$) and a significantly longer time alert in stage IV (W = 8946.5, $p = 0.006$) than the overall mean (**Figure 4.6**). As with Resting, time spent Alert was found to be influenced by date, where Alert was found to be highest at the mid-point of the season as indicated by the negative beta parameter for both terms of the polynomial relationship (**Table 4.4C**). A *post-hoc* analysis led to the removal of all extrinsic variables as well as all intrinsic variables with the exception of pup stage in predicting time spent Alert (see **Appendix Table A4.3a**, **b**). After reduction, models containing rMSSD, date, and pup stage carried over 98% of the overall model weight. Together, these models had an evidence ratio of 75.92 over the null model (**Table 4.3C**). Beta estimates for top models predicting time spent Alert were model averaged across all top models and can be found in **Table 4.4C**.

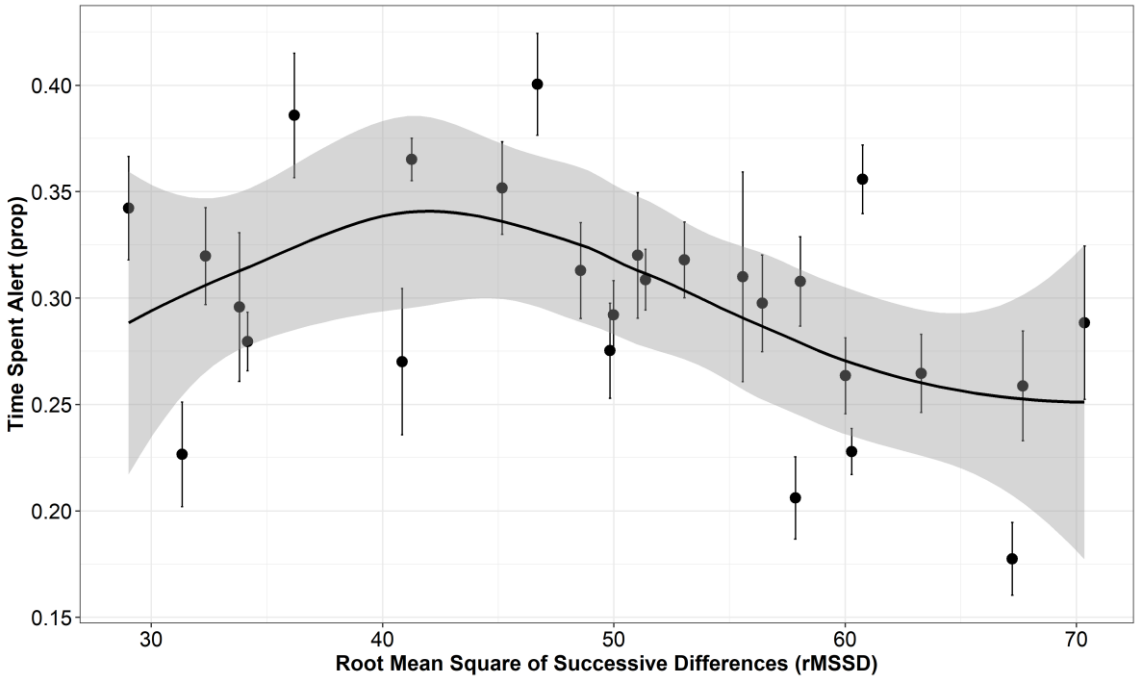


Figure 4.5: Scatterplot of time spent Alert as a function of the root Mean Square of Successive Differences (rMSSD), a measure of heart rate variability, in female grey seals. Point values represent the mean time spent Alert by each female, with standard error represented by the black lines. The black line represents a loess-smoothed trend line with 95% confidence intervals around the line in grey.

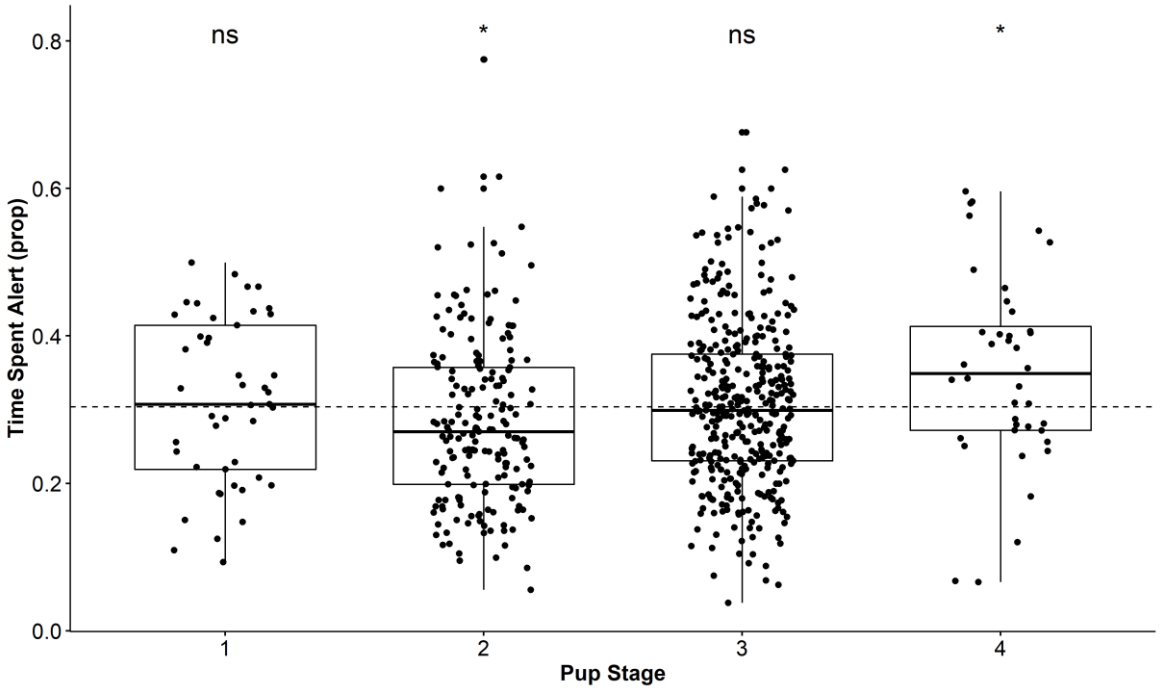


Figure 4.6: Boxplot of time spent Alert as a function of pup developmental stage. The dashed horizontal line represents the grand mean of time spent Alert. Female spent significantly less than average time Alert when pups were in stage 2, while spending more time Alert late in lactation when pups were at stage 4 as determined by a Wilcox test for each pup stage (indicated by * or ns above each boxplot).

4.3.4 – Interannual and individual consistency

A total of 7 females were recaptured between the 2015 and 2016 seasons. Using likelihood ratio tests (LRT) over 500 bootstrapped samples, very little variance was explained in time spent Resting, nor found to be significantly repeatable within individual ID (Variance = 0.3 ± 0.9%; *adj*-R = 0, $p = 0.396$) or year (Variance = 0.6 ± 0.7%; *adj*-R = 0, $p = 0.262$). Similarly, variance with respect to ID and year explained little to no variance in time spent Alert and was found not to be repeatable. However, individual ID was found to explain 8.4 ± 7.4% of the variance with respect to time spent Presenting/Nursing, but with very low and non-significant repeatability (*adj*-R = 0.004, $p = 0.138$). There was no significant repeatability nor variance explained by year with respect to Presenting/Nursing.

4.4 – Discussion

For the first time, maternal behaviour over a complete multi-day period during lactation was analysed using accelerometry data in grey seals, focusing on three core behaviours that constitute 95% of a given activity budget. While time allocation does not represent an exact measure of energy expenditure, it does provide a useful proxy for how individuals balance conserving energy through inactive behaviours against those active behaviours that may lead on from remaining vigilant, such as aggression or fleeing. Time spent Resting and Alert appear to represent a key trade-off in female grey seals; both behaviours were best predicted by identical model variables with opposing effects. The resulting relationships indicated that these two behaviours are complimentary with regards to the sex of a pup, its developmental stage, date, and the female's resting HRV. Significant differences were also found between pup sexes as well as between day and night periods with respect to these two behaviours. Time spent Presenting/Nursing, on the other hand, had no apparent link to any of the heart rate variability metrics tested. Counter to initial hypotheses, mothers did not differ significantly between male and female pups in time spent Presenting/Nursing, though they did tend to nurse

significantly longer overnight. Presenting/Nursing did vary significantly across the seven locations on the Isle of May in the current modelling framework.

4.4.1 – Trade-offs for conserving and expending energy

Given the evidence presented here, it appears that a trade-off in the conservation of internal resources through Resting and appropriately expending energy while Alert represents a key trade-off in time allocation over the course of lactation for grey seals. Interestingly, these two behaviours appeared to be subject to the changing density across the colony, as indicated by the opposing relationships between date for Resting and Alert. The population of the Isle of May, like many other breeding rookeries, follows a bell curve of increasing density towards the middle of the season around mid-November (Pomeroy et al. 2000b; a; Hall et al. 2001). While there are no daily estimates of colony density across the study period, the results with respect to trading off time spent Resting and Alert respond as one might expect. Females spent more time Alert and less time Resting mid-season when colony density is highest. This is in contrast to classic view of social vigilance in gregarious and herd-forming vertebrates, such as grey kangaroo (*Macropus giganteus*) among others, where female predator vigilance often tends to decrease with higher conspecific density (Loughry 1993; Beauchamp 2007; Pays et al. 2009; Favreau et al. 2015). Female grey seals, at higher densities, must remain vigilant to keep track of and protect their pups from other conspecifics and other threats (Pomeroy et al. 2000a; Twiss et al. 2003). Females were found to spend significantly more time Resting and less time Alert with pups in developmental stage II, while the opposite was found for pups in developmental stage IV as they approached weaning. During the early stages of development, pups are generally fairly immobile and do not tend to stray far from their mothers (Kovacs 1987). Milk fat content peaks during early development (Mellish et al. 1999a) and pups have the highest ratio of milk intake to mass gained during the early days of lactation (Iverson et al. 1993). Stage II of pup development (approximately day 5-9 of lactation; Woldstad and Jenssen

1999) may therefore be an important developmental milestone whereby pups must lay down their initial fat stores while both mothers and pups remain relatively inactive, maximizing mass-transfer. As pups approach stage IV near the end of lactation, mothers are approaching oestrus and likely therefore remain more vigilant for more frequent interactions with potential mates (Anderson and Fedak 1985; Tinker et al. 1995; Lidgard et al. 2001; Bean et al. 2004; Bishop et al. 2017). Previous work on grey seals in the UK indicate that females expend up to 10% more energy over gestation and lactation for male pups than female pups and spend significantly more time defending and nursing male pups than female pups (Anderson and Fedak 1987; Kovacs 1987). While it was found that grey seal mothers spent significantly more time Alert and significantly less time Resting with female pups than with male pups, it is unclear how much regional differences influence patterns of maternal investment, but the results presented here indicate that populations may experience different pressures on time allocation with regards to pup sex.

Trade-offs between the acquisition and conservation of resources against vigilance and anti-predatory behaviours have been examined in a variety of predator and prey contexts in terrestrial mammals, with varying degrees of density-dependence identified (Caro 1987; Burger and Gochfeld 1994; Hunter and Skinner 1998; Pangle and Holekamp 2010). In herding prey species, higher conspecific density can mean that certain individuals are able to spend less time Alert for predators, devoting a greater proportion of their time activity towards foraging or maternal care (Burger and Gochfeld 1994; Hunter and Skinner 1998). These trade-offs are also applicable where individuals must strike a balance between the energetic cost of foraging as a function of distance and search time and the acquisition of energy upon locating sufficient resources in the environment (Hill et al. 2003; Lagarde et al. 2008; Therrien et al. 2008; Watanabe et al. 2012; Patrick et al. 2014; Battaile et al. 2015; Flack et al. 2016; Costelloe and Rubenstein 2018). While herding animals benefit from decreased vigilance in large groups,

higher conspecific densities may mean that forage resources may be depleted more rapidly (Lindstedt and Boyce 1985; Festa-Bianchet et al. 2000). These examples principally illustrate the trade-offs and relationships between conserving or acquiring energy and expending energy to ensure survival. Female grey seals have been shown to have poor pupping success following a year with exceptional resource output into a larger pup (Pomeroy et al. 1999). Balancing time spent Resting and Alert may be key to lactating grey seals as they attempt to optimize resource availability for her current offspring while minimizing the impact of the associated longer-term costs on the next breeding cycle, though the actual energetic costs of engaging in these two behaviours is currently unknown. Tracking these behaviours across years may reveal consistent mothering strategies that may relate to different ways of managing these trade-offs with respect to ultimate fitness outcomes.

Very little work to date has focused on differences between day and night behaviour in grey seals. An early study by Anderson (1978) concluded that behaviour did not differ significantly between day and night; only the frequency of 'looks' was significantly higher during day and twilight hours (Anderson 1978). More recently, a few studies have found that females rest significantly more and were alert significantly less during night time hours, though the duration of night observations varied (Culloch et al. 2016; Fraser et al. 2019). Alert and other vigilance-type behaviours likely decrease at night due to a decrease in visual acuity, relying more on other sensory inputs to maintain contact with their pup and to intercept incoming threats (Schusterman and Balliet 1971; Schusterman 1974; Culloch et al. 2016). This may explain in part why behaviour has shown poor links to mass-transfer efficiencies when only using daytime activity budgets (e.g. Mellish et al. 2000). Accelerometers have opened the door to exploring the behaviour of many nocturnal species (e.g. Byrnes et al. 2011; McClune et al. 2014), and have even been used to determine the effects of artificial light pollution on changing the behaviour of diurnal species (e.g. peahens *Pavo cristatus*; Yorzinski et al. 2015).

If seals are exploiting night as a means of energy conservation and rebalancing time-activity trade-offs because of decreased visual acuity among conspecifics, artificial light pollution may impact this. While the Isle of May is largely undisturbed by human impact, encroaching human settlements towards mainland breeding sites may alter the night time activity in grey seals, especially for areas of newly formed breeding colonies and more ephemerally populated haul-out sites along the coasts of the UK.

4.4.2 – Stress-coping styles

Several measures of variability were extracted from heart rate data measured in grey seal mothers through lactation. While the effects of several aspects of resting HRV were tested, only a single measure was found to have extensive support in model evidence. The HRV measure rMSSD describes the norm of successive differences in inter-beat intervals, measured in this case during a period where the female was largely resting. This long-term measure of heart rate variability in the time-domain has been linked to a continuum of stress-coping styles in laboratory experiments, also known as the pro-reactive spectrum (Koolhaas et al. 1999). Individuals that display low HRV tend to be those classed as proactive and exhibit stereotypical, routine action patterns in a variety of contexts, while individuals that tend towards higher HRV show a more reactive coping style, where environmental inputs tend to elicit and dictate the responsiveness to a higher degree than internally regulated control (Coppens et al. 2010). Prior studies on grey seals have shown consistent female differences with respect to stress reactivity with the use of an acute novel stimulus (Twiss et al. 2012b; a). Given that rMSSD was found to be the best explanatory variable for time spent Alert and Resting, differences in whole animal stress-coping styles is suggested to be an important factor defining whole activity budgets in a wild mammal, rather than individual inputs of nervous system control as other HRV metrics were not retained. A lack of relationship to mean-f_H also supports the idea that individuals who are restricted in their daily energy expenditure should be

trading off activity energy with other background sources of energy expenditure and should have no relationship between activity and daily energy expenditure over time (Portugal et al. 2016; Careau 2017). Interestingly, no single measure of HRV could be found to predict time spent Presenting/Nursing, further supporting the notion that time spent in this behaviour is inflexible during lactation. Logically, a long-term measure of heart rate variability should be a good predictor of these differing activity budgets given the time-scales considered within this study. A lack of any relationship between other short-term measures of heart rate variability further supports this notion that innate differences in stress-coping style dictate the management of these chronic stressors over time. The other heart rate measures tested here, describing the differential inputs of sympathetic and parasympathetic, are likely to be better indicators of acute responses to stress, such as handling events (Mohr et al. 2002; Carravieri et al. 2016), or novel behavioural tests (e.g. Twiss et al. 2012b; a). Within these differing stress-coping styles, how these trade-offs in behaviour are expressed may dictate differential investment of partitions of energetic resources in order to maintain constant energy usage, but should to be investigated further.

4.4.3 – Variability in nursing effort

Intuitively, the drive to keep Presenting/Nursing effort consistent across lactation should be paramount to a female in order to maximize the likelihood that energy can be transferred to the pup. While the number of nursing attempts, or time spent Presenting/Nursing here, is not an accurate measure for amount of energy transferred (Cameron 1998), a theoretical minimum in Presenting/Nursing effort must exist and is likely strongly selected for in these seals. Likewise, seals should not overexert their energy output during lactation, as they risk jeopardizing subsequent breeding attempts (Pomeroy et al. 1999; Bubac et al. 2018; Desprez et al. 2018). Given that no evidence was found for external pressures, beyond colony location, or intrinsic traits acting upon time spent Presenting/Nursing, the results presented here

may indicate that this behaviour is highly fixed and selected for in female grey seals. Overall pup survival is highly dependent on the mass at which it is weaned (Hall et al. 2001; Bowen et al. 2015). Outside of sex-specific differences in survival, larger pups tend to lose fat at a slower rate during the extended on-land fast that follows the nursing period and will therefore likely still have plenty of fat stores to maintain themselves during their first forays into diving and foraging after departing their natal colony (Ortiz et al. 1978; Worthy 1991; Bennett et al. 2007; Bowen et al. 2015). To meet these ultimate fitness outcomes, females are likely less able to be flexible in trading-off time spent Presenting/Nursing with other behaviours over lactation in order to maximize energy transfer to her pup. With the use of accelerometers, it was found that females will often nurse significantly more overnight. Presenting/Nursing more overnight may help to stabilize this maternal effort over time, especially when social and aggressive interactions may limit the amount of time during the day that females can nurse.

The greatest source of variability in time spent Presenting/Nursing was found in the different sub-regions of the island. Several important factors may be at play in this situation. The seven locations on the colony (**Figure 4.1**) vary dramatically with respect to their topographical features and access to freshwater features. Access to freshwater has been shown to be an important factor in pupping site selection in grey seals (Twiss et al. 2000; Redman et al. 2001; Stewart et al. 2014). Phocid seals will use thermal windows in their blubber layer to thermoregulate, shunting blood to the surface to dump heat into the surrounding medium (Mauck et al. 2003). The thermal conductivity of water is 25 times greater than air, especially with the additive effects of evaporative cooling (Schmidt-Nielsen 1997). The thick blubber layer of all phocids complicates the normal avenues of thermoregulation and females therefore will often use freshwater pools to achieve the benefits of evaporative cooling and heat lost through water (Rommel et al. 1995; Boily and Lavigne 1996; Twiss et al. 2002; Khamas et al. 2012; Paterson et al. 2012). With the increased metabolic demands of lactating while fasting,

female grey seals likely prefer access to freshwater, not only as a means of thermoregulation, but also as a means to drink to meet water demands normally met from food (Stewart et al. 2014). Areas with the least access to freshwater pools tended to have females that spent significantly less time Presenting/Nursing on average, including the locations of Cross Park (XP), Tennis Courts (TC), and Rona Top (RT), than those containing relatively stable freshwater sources like Karren's Gully (KG) and Loan Road (LR; **Figure 4.1**). While not investigated in this study as locomotion-type behaviours were poorly identified by accelerometers (**Chapter 3**), it is more than likely that females with poor access to freshwater sources spent more time travelling to and from these pools to maintain their thermal and physiological balance, thus trading-off time to conserving energy. Some females may simply go without drinking, favouring lower activity levels at the expense of burning more internal resources to free up body water (Maltz and Shkolnik 1980; Schmidt-Nielsen 1997; McCue 2010). In either case, these two trade-offs may still result females using more energy over time, potentially tipping them beyond their physiological limits.

The area known as Tarbet Hole (TH) had an overall lower mean Presenting/Nursing proportion that the grand population mean, but with a high degree of variability. This is likely due to differences in access to a single large pool located in the centre of a densely populated area, but was not directly assessed in this study. Several large females, such as '45447' whose estimated post-partum mass was well over 200 kg (represented in the top quartile of all females in this study), were found to remain close to this pool of water and did not move in response to other females seeking out this water source, often engaging more in aggression-type behaviours towards females seeking access. This effect of apparent dominance may also vary with age. Locations with the lowest rate of Presenting/Nursing are generally more peripheral and seem to be populated with younger individuals, based on their small size. Some evidence in western Atlantic grey seals suggest that boldness, one of the hallmarks of individuals towards the

proactive end of the stress-coping styles spectrum, increases with age; younger females are on average less bold than older and more experienced females across a colony (Bubac et al. 2018). Older females on the Isle of May might be more bold and able to take advantage of prime breeding locations with access to freshwater. Since age cannot be accurately determined for most individuals without the extraction of a tooth, those few females with estimated ages in the current study having been branded as pups indicate that younger individuals closer to age at first reproduction were deemed as such due to their smaller overall body size (Pomeroy et al. 1999; Bowen et al. 2006). As female size increases and plateaus in the years following age at first reproduction, larger individuals may be able to access better areas of the colony (Bowen et al. 2006). Differences in location, and subsequently the amount of time spent Presenting/Nursing young, could be linked to water management or age related experience, but needs to be investigated further. Age- and location-specific effects on Presenting/Nursing also seem to be supported by the high degree of variance explained by individual ID between years (**4.3.4**). Age structured modelling of maternal effort, more specifically in relation to Presenting/Nursing effort investigated here, over time may reveal differences and the development of maternal strategies.

4.4.4 – Environmental considerations

Surprisingly, no support was identified for extrinsic environmental variables influencing behaviour in the current investigation. While all models containing weather variables were ultimately removed, it is most likely that the weather variables included were too coarse, both spatially and temporally, to register as a driver of time spent in any behaviour over each day and night period (Culloch 2012). Environmental variables are likely more important in dictating behavioural decisions on a much finer scale and should be investigated further. Thresholds associated with thermal tolerance have been observed to drive certain individuals to seek out water in order to thermoregulate, and is a commonly observed

behavioural mechanism in many pinnipeds (Gentry 1973; Schmidt-Nielsen 1997; Twiss et al. 2002; Wolf et al. 2005). Again, locomotory behaviours were poorly identified (**Chapter 3**) and prevent an accurate discrimination of movement across the colony to and from freshwater sources, without the aid of additional sensors like GPS or magnetometers for dead-reckoning. Outside of effects related to age and boldness scores, some evidence of thermal stress in grey seals has already been documented and is likely to be a continued driver of behaviour and interactions with freshwater sources as the climate is projected to warm and dry in the UK (Twiss et al. 2002; Jenkins et al. 2008). The current study may highlight a potential effect of the interaction between stress-coping styles and the environment. Proactive individuals have already been shown to be better suited to stable conditions and are likely to be the most resistant to change (Coppens et al. 2010). Reactive individuals, on the other hand, are likely best suited for handling an increasingly variable climate by their ability to be more resilient and flexible with fluctuating environmental cues from year to year, though responses between individuals are likely to be more variable overall (Coppens et al. 2010; Koolhaas et al. 2010).

While there was no direct link made to this idea of behavioural flexibility and ultimate fitness outcomes in this chapter, one would predict that some reactive individuals in a changing and more variable climate may longitudinally have a higher average fitness than most individuals toward the proactive end of the spectrum. While it is unclear to what degree these personality traits are heritable, reactive individuals likely persist in the population, not because they may perform poorly in the short-term, but because their behavioural flexibility balances short-term losses to long-term gains in this iteroparous species (Phillimore et al. 2016; Tansey et al. 2017; Chmura et al. 2018). Proactive individuals perform better in more stable years of breeding, while reactive seal mothers may perform better in breeding seasons where environmental conditions are more variable. A variety of stress coping styles within a population can buffer populations to changes in conditions over time in species that can breed

multiple times over their reproductive life, allowing for population-level resilience in pup-production within a fluctuating environment.

While the current study likely offers the finest scale effort to study behaviour to date, trade-offs between years could not be confirmed for their consistency as no significant repeatability could be identified as a result of small sample sizes. Simply allocating activity budgets into day and night blocks appeared to be too coarse to find significance within and between individuals. It is likely that through investigating decision-making processes on a finer temporal scale, especially with regards to extrinsic drivers (e.g. how hourly temperature may dictate activity levels), that one may find individual behavioural decisions on whether to be inactive (conserving resources) or active (expending resources) to be more repeatable. Consistent individual differences are most often found within and across specific contexts, measured across much finer time-scales than were currently investigated (e.g. Coleman and Wilson 1998; Dall et al. 2004; Twiss and Franklin 2010). While significant changes in female behaviour associated with the sex of her pup, its developmental stage, location on the colony and stress-coping styles between day and night were found, other extrinsic factors are more likely to be acting upon the minute-to-minute decision making process. I would also expect the effects of stress coping styles to become more prevalent on such a fine-scale, ultimately displaying differential patterns of energy usage over time.

4.4.5 – Conclusions

Using the high-resolution data from accelerometers, this thesis was able to identify behavioural trade-offs between rest (Resting) and vigilance (Alert) in a capital breeding pinniped. Presenting/Nursing as a behavioural state appears to be more set within an individual, varying instead across the colony. Stress-coping styles, measured through the metric rMSSD, were also found to have an important influence on behaviour throughout the

duration of lactation and appears to be a key modifier of these behavioural trade-offs. As a result, one can conclude that females appear to only be flexible in a few portions of their activity budget in order to respond to conspecifics and other intrinsic drivers over such a constrained lactation period while maintaining enough resources to successfully complete lactation the next season. Incorporating the dynamics of these trade-offs on whole activity budgets as well as the modulating effect of stress-coping styles will enhance our understanding of how individuals optimize energy usage trajectories over time.

Chapter 5:

Behavioural energetics of lactation: Investigating individual differences and energy management strategies in relation to fitness outcomes and environment

Manuscripts from this Chapter:

SHUERT, C. R., L. HALSEY, P. P. POMEROY, AND S. D. TWISS (*in prep*) Envelopes of energy management: Defining the limits of plasticity in energy management in a capital breeder. Ecology Letters. [*Author Contributions*: CRS collected the data and performed the analyses with support from LH, SDT and PPP. CRS wrote the paper with input from all co-authors]

SHUERT, C. R., P. P. POMEROY, AND S. D. TWISS (*in prep*) Climate impacts on metabolic scope and plasticity during lactation in a free-ranging terrestrial breeding pinniped. [*Author Contributions*: CRS collected the data and performed the analyses with support from SDT and PPP. CRS wrote the paper with input from all co-authors]

SHUERT, C. R., P. P. POMEROY, AND S. D. TWISS (*in prep*) Heart rate variability impacts energy management during lactation: Evidence for pace-of-life in a wild pinniped? [*Author Contributions*: All authors collected the data and drove development of the manuscript. CRS led writing the paper with input from all co-authors]

5.0 – Abstract

Lactation is an energetically demanding period for any female mammal. Females must carefully balance maximizing the amount of energy passed to their dependent offspring, while still maintaining enough energy stores to support themselves. Capital breeding systems are found in most phocid seals, whereby females must sustain themselves and their offspring over the lactation period while fasting exclusively on energy reserves acquired prior to breeding. This chapter sought to holistically describe energy usage and management for individual grey seal mothers for the core duration of lactation using accelerometry and heart rate methods. More specifically this included describing how activity levels change over time, how extrinsic drivers such as ambient temperature may dictate activity and energy, how grey seals manage energy, and ultimately how individual differences in stress-coping style drives energy management and short-term fitness outcomes. Grey seal females appear to remain inactive for the majority of the breeding season as a means of conserving energy, as accelerometry-derived activity did not directly correlate with absolute energy usage. Accelerometer-derived activity bins and aspects of heart rate revealed that stress-coping styles play a significant role in dictating the amount of energy transferred to her pup, but at different costs to the individual. As a whole, grey seal females cope with periods of high background metabolism output by minimizing energy spent on activity, thus maintaining a reasonably consistent daily energy expenditure. The thermal environment also appeared to play a significant role in energy management; individuals appeared to minimize background processes in order to compensate for increasing thermoregulatory burdens. This has been further supported by the results presented here showing that compounding effects of stress management have a direct influence on energy management and energy usage on a system where the margin of error is slim for effectively transferring energy to offspring.

5.1 – Introduction

5.1.1 – Lactation and energy allocation

Several theoretical models have been hypothesized to characterize energy management in mammals. Careau and Garland Jr. (2012) proposed four models illustrating energy management strategies known as the increased-intake, compensation, independent, and substitution model. These serve as a hypothetical framework relating how individuals may balance metabolic demands with the energetic expenditure associated with daily activities. Lactation is a defining characteristic of mammals and likely the most energetically demanding period for any female. Females must efficiently mobilize fat and protein stores into a lipid rich secretion from mammary tissue to supplement their developing offspring post-partum. The energy management strategy of any female mammal over the duration of lactation likely falls within either the increased-intake or the compensation model as a way to handle the increased demands of milk production. The increased-intake model in this lactation context, as the name suggests, proposes that a female must meet the increased metabolic demands of lactation, and possibly an increased daily energy expenditure, by continuously supplementing energy reserves with food (Careau and Garland Jr. 2012). The compensation model, on the other hand, proposes that a female must operate within a fixed set of energy reserves to balance energy spent on metabolic activities and daily energy expenditure (Careau and Garland Jr. 2012).

Lactation is typically characterized by a general increase in basal metabolic rate, enhanced enzyme activity, and a rapid turnover of metabolites into milk energy output (Tedman and Green 1987; Schweigert 1993; McLean and Speakman 1999; Mellish et al. 2000). During this period, females must carefully balance maximizing the amount of energy passed to the dependent offspring, while still maintaining enough stores to support themselves. The increased-intake and compensation energy management strategies mirror similar ecological concepts of lactation strategies, namely income and capital breeding, respectively

(Stephens et al. 2009). Income breeding, often found in Otariids, indicates that individuals will supplement their energy stores during breeding by embarking on periodic foraging trips by either leaving their pup on shore or by having an increased dependency period where the pup stays with the female (e.g. McDonald et al. 2009). The capital breeding system is found in most Phocid seals including grey seals, where breeding females must sustain themselves and their offspring exclusively on energy reserves acquired prior to the fasting lactation period (e.g. Schulz and Bowen 2005). Given the constraints of this capital breeding system, energy must be allocated to several key areas during lactation within the framework of this compensation model. Females must have some energy stored to maintain themselves during the increased metabolic output that lactation requires. Resting metabolic rates have long been defined as the mass-specific rate of oxygen consumed to sustain an individual who is unmoving, non-absorptive, thermoneutral, and not reproductively active (Kleiber 1961). Female phocids typically have some of the highest energy demands of any mammal during lactation, upwards of three times the maintenance requirements of what would be predicted from their estimated mass-specific metabolic rates, and over seven times the predicted level of total daily energy expenditure (Boily and Lavigne 1995; Schmidt-Nielsen 1997; Mellish et al. 2000). This means that there must be a minimum energy store required for females to sustain themselves during the high energy throughput of lactation.

Along a similar vein, female pinnipeds will undergo an embryonic diapause in order to maintain a synchronous breeding structure, pushing back implantation several months or weeks following successful copulation (Boyd 1998). This embryonic diapause is often found to align with the termination of moult, where females must undergo a second fasting period while on land, likely before they have fully recovered body stores following lactation (Boyd 1991). It is during the moult that females may be in the poorest condition. Female phocids likely have a threshold of minimum energy reserves at this time point before undergoing such a demanding

period of life history, and may be a reason underlying early termination of pregnancies (Boyd 1991). What this threshold may be, however, is poorly understood and a similar mechanism is likely at play during lactation and may be one of the reasons for the early cessation of nursing observed in some individuals (**Table 2.1**). Given that female phocids are iteroparous, forgoing pregnancy or terminating lactation early in years where body or environmental conditions are poor may serve to minimize the long-term costs to the female within the compensation model, while maximizing lifetime reproductive output (Pomeroy et al. 1999; McMahon et al. 2016; Desprez et al. 2018).

Females should attempt to maximize the energy passed on to offspring by prioritizing the allocation of non-maintenance energy outputs into milk production in a compensation framework, while minimizing the negative effects to the self. Offspring body size in many species has been found to be a very robust predictor of survival, both in the short-term following weaning and longer-term recruitment into adult age classes (Loison et al. 1999; Harding et al. 2005; Bennett et al. 2007; Ozgul et al. 2010). A shortening of this lactation period within phocids exponentially increases the importance of this aspect of energy allocation, reflected in a logarithmic increase in milk fat content as a function of decreasing lactation duration (Oftedal et al. 1996). True seals, phocids, have some of the shortest lactation periods of any eutherian mammal (Schulz and Bowen 2005). The shortest lactation belongs to that of the hooded seal (*Cystophora cristata*), lasting only an average of 4 days, where females may produce up to 10 kg/day of milk (Iverson et al. 1995; Mellish et al. 1999b). Energy outputs into milk from a female phocid would likely fall into energy allocated for metabolic activities, as has already been shown in grey seals (Mellish et al. 2000), limiting energy left over for daily activities. While the evolution of such tight constraints likely arose from the ephemerality of the pack ice which most true seals inhabit during breeding, this shortened

period of lactation in grey seals leads to a very intense period of metabolic compensation and energy expenditure that females must maintain (Schulz and Bowen 2004, 2005).

Since lactating phocid seals have a fixed energy reserve, energy expended through other behaviours should be reduced in order to operate with the increased metabolic demands of lactation (background and milk energy expenditures together) while fasting (McLean and Speakman 1999; Mellish et al. 2000; Careau and Garland Jr. 2012; Collins et al. 2016). This area of energy allocation must balance against maintenance expenditures, reflected by a negative relationship between activity and background energy under constant total daily energy expenditure. Phocid seals are often observed to limit their overall activity levels over lactation as an energy saving mechanism (Anderson and Fedak 1987; Thompson et al. 1994; Hood and Ono 1997; Mellish et al. 2000). The amount of time spent nursing is obviously linked to maximizing the transfer of lactation expenditures to offspring. However, the expression or depression of other behaviours may dictate how efficiently a female can operate within this small remaining pool of energy available without dipping into energy stores needing to be allocated elsewhere. It is unclear how small alterations in the remainder of a female's activity, either due to consistent differences in suites of behaviour or daily fluctuations in time allocation, will impact her overall energy reserve balance.

5.1.2 – Estimating energy usage over time

Activity-specific energy usage has been measured in several ways over the last few decades. Typically, energy usage has been directly or indirectly measured through the use of respirometry techniques by measuring the influx of oxygen and outputs of carbon dioxide to directly quantify the metabolic activity over time (Costa and Kooyman 1982; Ward et al. 2002; Yeates et al. 2007; Wright et al. 2014). These methods have made it possible to directly measure the volume of oxygen used by an organism both during periods of rest as well as the

energy used during different exercise related trials, such as diving in a pool or running on a treadmill (Green et al. 2001; Ward et al. 2002; Wilson et al. 2006; Fahlman et al. 2008; Rosen et al. 2017). Respirometry however, while likely to be the most accurate, is expensive to set up and often require captive individuals housed in a mesocosm facility, which can make the practice prohibitively expensive and logistically difficult to apply in a natural setting. Isotopically-labelled injectables are also a popular means to determine body composition and energy used over time (Reilly and Fedak 1990; Scrimgeour et al. 1993; Mellish et al. 1999b; Rutishauser et al. 2010; Rea et al. 2016). Doubly-labelled water is often used as an indirect method for estimating metabolism and metabolic energy usage by measuring the differential uses of isotopically-diluted hydrogen and oxygen in the body (Boyd et al. 1995; Sparling et al. 2008; Dalton et al. 2014; Elliott 2016), while singly-labelled isotopes, such as deuterium and tritium, can be used to estimate changes in total body composition associated with energy usage over time (Scrimgeour et al. 1993; Arnould et al. 1996; Bowen and Iverson 1998; McLean and Speakman 1999; Shuert et al. 2015b). Despite being more appropriate and applicable for field situations, isotopically-labelled water can still be prohibitively expensive and only give a brief snapshot of energy usage over a few handling events.

Advances in biologging technology have offered arguably cheaper alternatives to determine energy usage over time. Heart rate has had a long-established link to energy usage in association with both resting and exercise through the use of the Fick equation (e.g. Schmidt-Nielsen 1972, 1997). Outside of differences in blood oxygen stores within an individual, the rate of cardiac output should scale accordingly with the volume of oxygen consumed. Once species-specific rates of oxygen consumption are calibrated, the use of externally mounted heart-rate monitors should be a robust proxy for both the resting metabolic rate and activity-specific energy usage (Boyd et al. 1995; Webb et al. 1998; Wilson et al. 2006; Elliott 2016; Portugal et al. 2016). Accelerometers have also recently become popular tools for

determining activity levels, even allowing researchers to remotely extract a variety of specifically defined behaviours with varying degrees of energy usage associated with them (e.g. Wilson et al. 2006; Bishop et al. 2015b; Jeanniard-du-dot et al. 2016b). Accelerometers measuring activity through summed vectors of movement must be placed on the centre of mass in order to best estimate energy used over time, supposedly scaling appropriately with the overall work output of all muscles involved in movement, especially in the context of movement or exercise trials (Wilson et al. 2006; Halsey et al. 2009b, 2011a; Gleiss et al. 2011; Fossette et al. 2012; Miwa et al. 2015; Stothart et al. 2016). These methods have seen varied success in relating activity to energy budgets over time. Relationships of total oxygen consumption and work tend to grow with time which can easily lead to spurious correlations between measures of activity and metabolism, the so-called 'time-trap', as would be found by estimating total oxygen consumption using doubly-labelled water for a foraging trip (Halsey 2017; Ladds et al. 2017a). However, some studies have overcome this by combining heart rate and activity levels in order to build a robust model of the rate of energy usage for specific behaviours, also allowing for a measurement of internal state (e.g. Halsey et al. 2009a; Bishop et al. 2015b; Portugal et al. 2016).

5.1.3 – Factors influencing energy usage over time

There are many factors that may be acting upon an individual that likely drive their decision-making process with respect to activity levels over time. So-called energy trade-offs are often driven by external factors such as temperature, prey availability, and landscapes of fear (Gallagher et al. 2017). Energy trade-offs in lactation are most often explored in foraging contexts in both terrestrial environments and those marine mammals exhibiting income breeding. Often operating within the increased-income energy management framework, those individuals classified as income breeders are discussed as trading off time-activity for foraging (acquiring energy) and provisioning young (expending energy; Boyd 1998; Gaillard et al.

2000; Bowen et al. 2001; Witter et al. 2012). Under nutrient limitation experiments, female ungulates are often seen to prioritize their own energy needs over those of their offspring (Therrien et al. 2008). In some cases, these external pressures may be beyond a female's energetic capacity and plasticity to handle, extending beyond the desired physiological scope that these trade-offs in energy management operate in.

While a fasting lactation on land, as seen in grey seals, means that prey availability and predator avoidance do not exert a great amount of influence on individuals at this stage of life-history, external pressures like temperature, and subsequently thermal tolerance, likely play a bigger role in dictating the priorities of energy management. A changing climate has already been shown in a wide variety of vertebrates to not only push some individuals outside of their thermal neutral zone, but also introduces an increasingly important additional cost to balance within an individual's activity and energy budget (Trites 1990; Twiss et al. 2002; Hill et al. 2003; Beentjes 2006; Rutishauser et al. 2010; Fossette et al. 2012; Moses et al. 2012; Goulet et al. 2017; Udyawer et al. 2017; Payne et al. 2018). While thermal tolerance, as measured via thermal neutral zones, has been studied extensively in non-reproductive individuals across multiple age classes, little to no work has been done to investigate thermal tolerance in a reproductively active female grey seal (Boily 1995; Schmidt-Nielsen 1997; Rey et al. 2015). The vastly increased metabolic compensation of lactation coupled with large body mass and highest annual fat content may mean that female grey seals have a lower thermal tolerance than previously measured in non-reproductive individuals and may be at risk of physiological stress.

Recently, individual differences in stress-coping styles have been shown as a potential explanatory variable for individual variability in behaviour as well as inherent differences in metabolic rates and other physiological processes (Careau et al. 2008; Houston 2010; Careau 2017). One method of measuring stress-coping styles is through the use of heart rate monitors

to measure differences in resting heart rate variability (von Borell et al. 2007). Heart rate variability is becoming a popular tool with which to measure stress in a variety of situations in mammals (de Jong et al. 2000; Mohr et al. 2002; Schmidt et al. 2010; Liu et al. 2014). Among the metrics of heart rate variability, rMSSD, quantifying the variability in successive inter-beat intervals, has been a popular method of differentiating stress-coping styles in the literature (Reefmann et al. 2009; Schmidt et al. 2010; Briefer et al. 2015), and was already found to be an important predictor in whole activity budgets in grey seals (**Chapter 4**). Individuals with lower HRV in heart rate are typically regarded as proactive and are generally fixed in their behavioural patterns, responding less to environmental cues, and may have higher than average metabolic rates (Careau et al. 2009; Coppens et al. 2010). Individuals with higher HRV, on the other hand, tend to respond to a much greater degree to environmental cues, remaining behaviourally flexible to an unstable environment, but often with lower than average metabolic rates (Careau et al. 2009; Coppens et al. 2010). While these fixed or flexible behavioural patterns have been linked to underlying neuroendocrine functions and various physiological pathways, it is unclear to what degree this spectrum of stress-coping styles may affect energy usage over time and if any measurable fitness consequences exist in a wild context. Behavioural indications of consistent and repeatable stress reactivity in female grey seals has already been demonstrated to have a potential effect on the variability of fitness outcomes (Twiss and Franklin 2010; Twiss et al. 2012a; b). With respect to energy usage, the tight constraints of a compensation model may mean that differences in stress-coping styles in a wild population have a much more profound effect on fitness outcomes than observed in a laboratory setting (e.g. Monestier et al. 2015). Various measures of heart rate have recently been shown to be a reliable, non-invasive indicator of metabolic rate (minimum heart rate), daily energy expenditure (mean heart rate), and activity-specific energy usage (the difference between the two) in a variety of taxa (Portugal et al. 2016). Combining proxy measures of

energy usage (heart rate) and stress-coping styles (heart rate variability) may provide links between the consequences of personality and energy management on fitness outcomes.

5.1.4 – Study goals

The aim of this chapter was to describe individual differences in energy usage in the female grey seal during lactation through the use of torso-mounted accelerometry and heart rate methods. The first aim of this chapter was to quantify how activity changes over time and the factors driving changes, specifically how fine-scale spatial and temporal changes in temperature may dictate activity levels over time. For the second aim of this chapter, combining the information from animal-borne heart rate monitors as a proxy for energy usage, energy management models were quantified in study females by examining the relationship between minimum and activity-specific energy usage. The ability of accelerometer-derived activity levels to predict energy usage was evaluated by regressing mean acceleration on mean heart rate (daily energy expenditure). In addition, the response of each of the three heart rate metrics for energy usage to different activity levels and temperatures experienced were contrasted and compared to further describe energy management in grey seals. The final aim of this analysis sought to explore how coping style (measured through rMSSD) may influence activity levels over time and impact the rate of mass lost and potential fitness outcomes, as measured through mass-transfer efficiency. The ability to characterize energy usage over time as well as the factors driving decision making would allow for the identification of critical periods in lactation and allow for the prediction of an individual seal's response to a changing environment.

5.2 – Methods

5.2.1 – Tagging and derivation of activity levels

Small data-logging accelerometers were placed on the torso of 34 female grey seals for the core duration of lactation (capture and tagging procedure outlined in sections **2.2** and **2.3** of the general methods; **Figure 5.1**). Accelerometers were sampled at 25 Hz at ± 2 *g* in 2016 (n_{ind} = 10) and 50 Hz at ± 4 *g* in 2017 (n_{ind} = 20; AXY-Depth, Technosmart Europe, Italy). In addition, 4 individuals were equipped with GPS-enabled accelerometers in the 2017 season (50 Hz ± 4 *g*; AXY-Trek, Technosmart Europe, Italy). Only those individuals that were sampled for a minimum duration of 6 days to capture the core of lactation were included in this chapter (total n_{ind} = 31). For the purposes of this analysis, only the vectorial dynamic body acceleration (VeDBA, **Eq 2-2**) and the smoothed vector of VeDBA (VeDBAs) were derived from the raw accelerometry data. These were then summed hourly and split into consecutive day and night periods (split by civil twilight as in section **4.2.1**), as day and night have already been shown to highly influence behaviour time-activity budgets in these females (**Chapter 4**). The mean of hourly summed activity (VeDBAs), a proxy for activity-specific energy usage, was then plotted for each day and night separately over the length of tag deployment to describe individual differences in trajectories of energy usage. Due to differences in sampling rates between years, accelerometry data for repeat capture females (n_{ind} = 6) in 2017 were down-sampled to 25 Hz in order to directly compare summed activity trajectories.

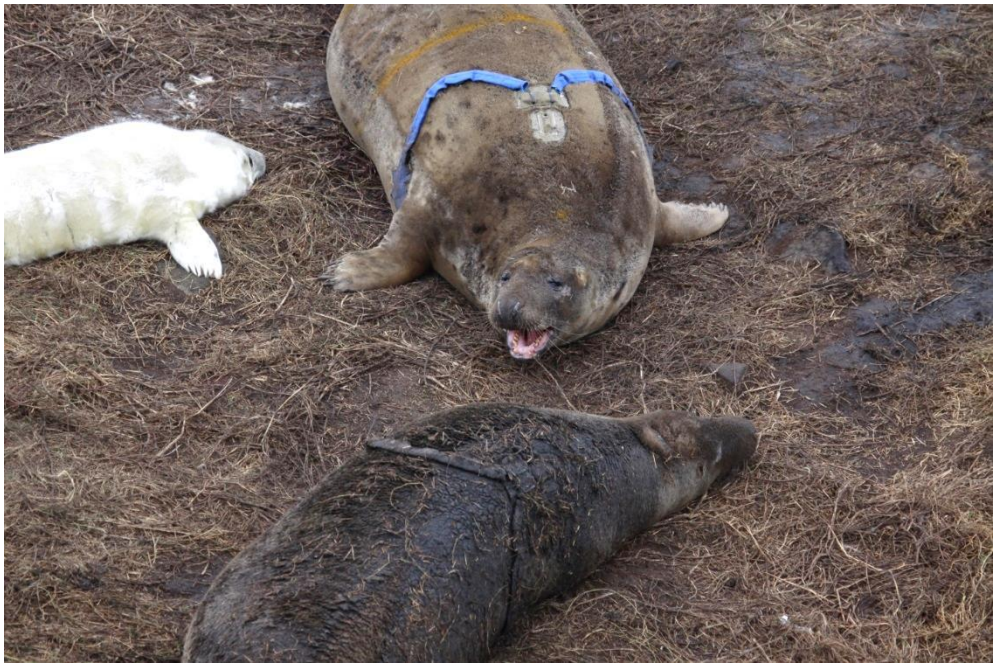


Figure 5.1: Example of accelerometer mounted between the shoulder blades, and heart-rate monitor attachment set up on two study females during the 2017 season (Photo: SD Twiss).

5.2.2 – Modelling activity and heart rate

In addition to accelerometers, study females were also equipped with heart rate monitor activity belts as described in section **2.3** (**Figure 5.1**). A sample of heart rate (beats min^{-1}) over resting and activity periods was possible for most individuals while accelerometers were deployed (2016 n_{ind} = 7; 2017 n_{ind} = 17). As a consequence of the field sampling protocol, sample sizes for modelling activity exclusively from accelerometers (loggers) differed from those including heart rate and heart rate variability measures (transmitters). Segments of heart rate data (n = 2,324 15-min segments) were time-matched to the raw accelerometer data to create summaries of heart rate and its variability across a variety of activity levels. Only those heart rate segments with less than 50% flats and stairs were included (as outlined in **2.3**). Minimum heart rate (min-f_H) was extracted as the lowest measured heart rate across all segments for a given day and was used as a robust proxy of basal metabolic rate for each individual. Mean daily heart rate (mean-f_H) was also extracted as the grand mean across all segments within a day and was used as an estimate of daily energy expenditure (Portugal et al. 2016). Activity-specific heart rate (also termed auxiliary heart rate; aux-f_H) was determined by

subtracting min-f_H from the mean-f_H and used as proxy for activity-specific energy expenditure for each day (Halsey et al. 2019). The slopes of the relationship between min-f_H and aux-f_H for each female were evaluated to quantify the presence of an energy management strategy in these grey seals (Careau and Garland Jr. 2012; Halsey et al. 2019).

The mean value of VeDBA as calculated over each 15-min period was binned into 3 levels of activity based on previous calculations of more specific behaviours in **Chapter 3**: inactive (comprised mostly of behaviours involving little to no movement; mean VeDBA less than 0.0275), low activity (observed in behaviours involving little movement while the animal is alert; mean VeDBA ranging 0.0275 – 0.05), and high activity (those behaviours where the animal is continuously moving, such as locomotion or aggression; mean VeDBA greater than 0.05). Daily min-f_H, mean-f_H, and aux-f_H were each modelled as a function of the proportion of time spent in each of the three activity states (measured as the proportion of 15-min segments in each state within a day). In order to investigate if a relationship between heart rate measures of energy expenditure and accelerometer-derived energy expenditure exists, mean VeDBA was regressed against mean-f_H.

5.2.3 – Effects of heart rate variability on energy usage and fitness outcomes

In **Chapter 4**, the heart rate variability metric rMSSD (Marchant-Forde et al. 2004), measuring the variability of successive inter-beat intervals, was found to be an important predictor of total activity budgets. Individuals with lower rMSSD values tend towards the proactive end of the spectrum and are relatively fixed in their action patters, while individuals with higher rMSSD values move towards being more reactive and tend to respond more to environmental cues (Koolhaas et al. 1999, 2010, 2011; Coppens et al. 2010). The relationship between heart rate variability (rMSSD) and time spent in each of the binned activity levels was explored as well as how differences in stress-coping styles may contribute to the ultimate

fitness outcome proxies, MT_{eff} (the proportion of female mass loss to pup mass gains) and mdml corrected for initial female mass (g kg^{-1} d^{-1}, see **2.7**; Wheatley et al. 2006). In addition, the relationships between rMSSD and the three heart rate metrics (min-f_H, mean-f_H, and aux-f_H) were also assessed. Due to issues with heteroscedasticity associated with the relationship between rMSSD and fitness outcomes, individuals were classified as having low variability if they had an rMSSD value less than 55 or as having high variability if above that cut-off. This cut-off was chosen as it reflected an inflection point in the relationship between rMSSD and activity budget parameters in **Chapter 4** (see **Figure 4.3** and **4.6**).

5.2.4 – External factors determining behaviour

The effects of localized temperature on the activity levels were investigated for these female grey seals during lactation. For the 2017 season, 8 small temperature loggers were deployed (iButtons; see **2.8**) across key geographic locations of the colony into the substrate, attached to small stakes in the ground (**Figure 5.2**). These loggers were configured to record the temperature to the nearest 0.5 ^{o}C every 30 minutes for the duration of the season. Brief spikes in temperature well above 18 ^{o}C were removed in order to exclude times where a seal was likely to be lying on the temperature logger, registering the surface temperature of an animal rather than the substrate. Missing values from the removal of outliers were replaced by imputation based on a running mean of prior temperatures. The effect of temperature on overall activity levels was explored as well as how maximum daily temperature may have an effect on estimated daily resting metabolic rate (min-f_H), daily energy expenditure (mean-f_H), and daily activity-specific energy expenditure (aux-f_H).

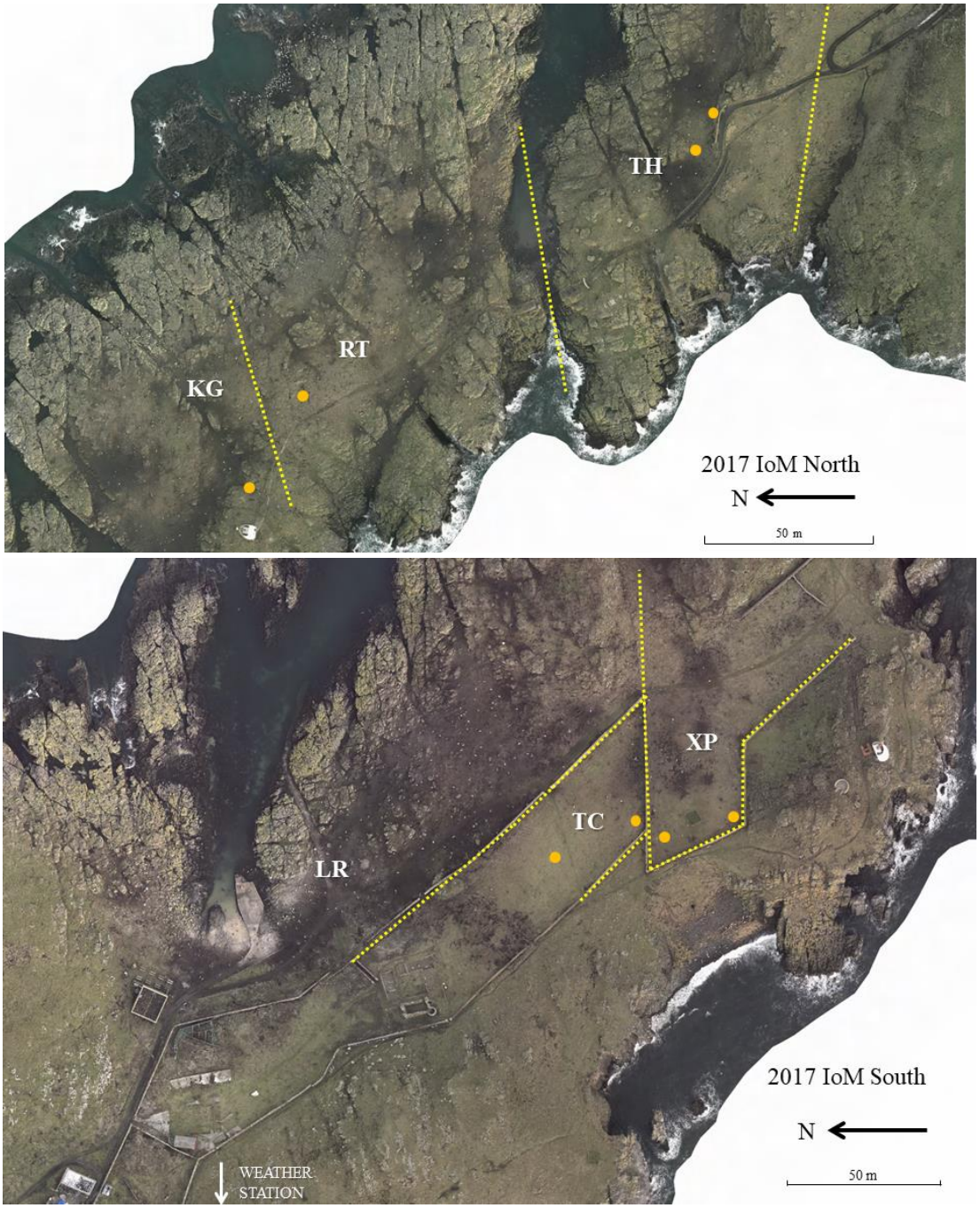


Figure 5.2: Locations of iButton thermologgers (orange dots) around the sub-locations (North to South, sub-locations outlined with yellow dotted line: Karren's Gully – KG; Rona Top – RT; Tarbet Hole – TH; Loan Road – LR; Tennis Courts – TC; Cross Park – XP) within the Isle of May.

A binomial generalized linear mixed effects model was used to predict the probability of being active as a function of temperature and day and night (dn) differences as a way to model the potential energetic consequences of temperature and to evaluate if, and where, a temperature-associated threshold for activity may exist, potentially indicating thermal stress.

Activity was extracted by classifying mean VeDBA over 30 min periods (to match the resolution of temperatures recorded) as either inactive ('0'; mean VeDBA < 0.0275) or active ('1'; mean VeDBA > 0.0275). Each individual was spatially matched to the nearest iButton temperature logger (**Figure 5.2**). Individual ID was included as a random effect. Models including the singular, additive and interactive effects of temperature and dn were competed against a null random effects model using AICc model selection methods (Burnham and Anderson 2002; Burnham et al. 2011). The relative effect sizes of variables and the log-odds of activity associated with temperature were evaluated.

5.3 – Results

5.3.1 – Changes in activity levels over time

Overall activity levels in lactating female grey seals, represented by daily means of summed VeDBAs (subsampled for 2017 to match the sampling rate of 2016), were found to be higher in 2016 than in 2017 (**Figure 5.3**). Across all females, daytime activity levels appeared to be lowest in the first few days measured in lactation up until about day 7, while night time activity levels seem more stable through the course of lactation, though sample sizes are low in early lactation (based on visual inspection, see wide confidence intervals). This is supported by the consistency of daytime activity levels between years for the majority of the 6 females captured in both study years (**Figure 5.4**). The pattern (shape of the curve) of activity during the daylight across lactation appears to be remarkably consistent between years, regardless of an increase in activity for most repeat capture females in 2016 (mean difference between 2016 and 2017 was -0.108 ± 0.067). Individual activity levels across night are much less consistent between years (-0.124 ± 0.071 between 2016 and 2017) and show overall lower activity than during the day (scales differ as night activity is summed over a greater time period; **Figure 5.5**).

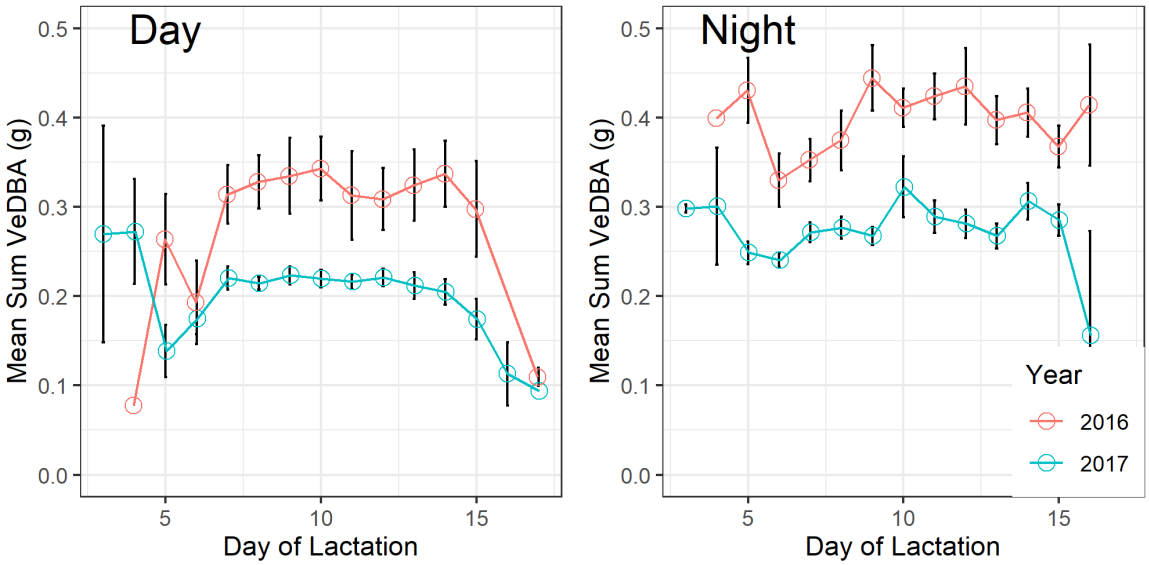


Figure 5.3: Interannual comparison of day and night activity levels with respect to the day of lactation in female grey seals. Activity level was calculated as the daily or nightly summed VeDBA as collected from torso-mounted accelerometers. In order to match the sampling frequency of 2016 (25 Hz, *n* = 8), the accelerometry data for 2017 (50 Hz, *n* = 23) was down-sampled by half. Day and night activity levels are plotted separately as activity overnight is summed over a longer time period during the autumn (approx. 16 hours). Open circles represent the mean of summed activity across all females, bars representing ± 1 standard error.

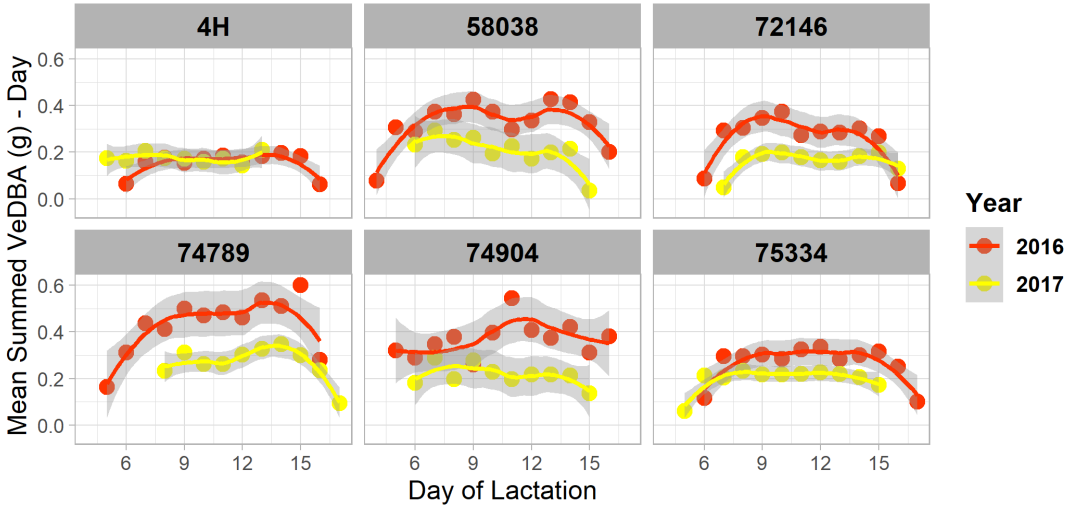


Figure 5.4: Day-time activity levels (mean summed VeDBA, *g*) for 6 repeat capture female grey seals across lactation. In order to be comparable, 2017 data was down-sampled by half. While activity was generally higher in 2016, females appear to show very consistent patterns in activity levels across lactation between years during daytime hours. Lines represent loess smoothed trends of each year (grey represents 95% confidence interval).

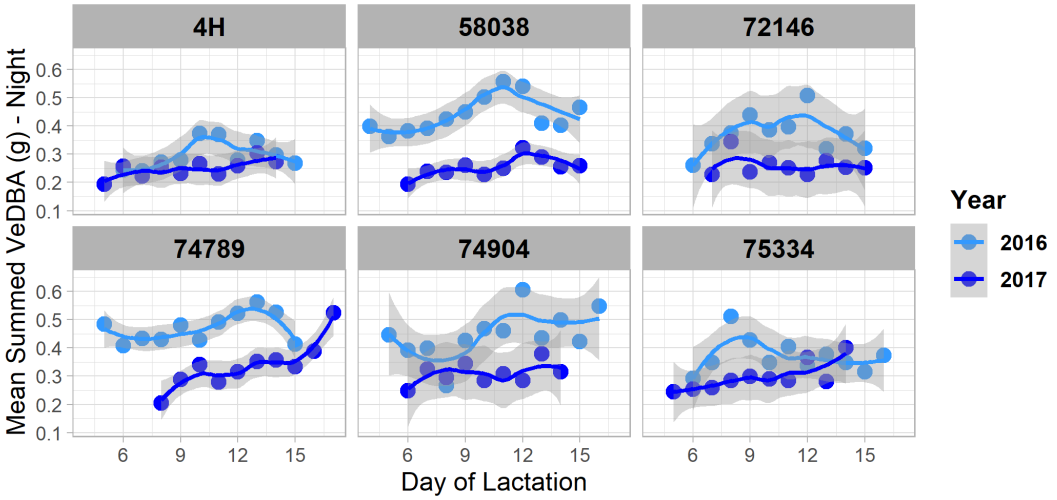


Figure 5.5: Night time activity levels (mean summed VeDBA, *g*) for 6 repeat capture female grey seals across lactation. In order to be comparable between years, 2017 data was down-sampled by half. Night time behaviour again was higher in 2016, but less consistent between years. Lines represent loess smoothed trends for each year (grey represents 95% confidence interval).

Overall, temperature was found to have a minor effect on activity levels during lactation. Using binomial generalized linear mixed effects models, extensive support was found for the additive and interactive effects of temperature and dn, carrying all of the model weight (**Table 5.1A**). While the effect size of day and night was the greatest, temperature still had a minor influence on activity levels (**Table 5.2A**). This was especially true with respect to the effect of an interaction between dn and temperature; higher temperatures during the day increased the probability of activity by just under 10% (**Figure 5.6**). In order to separate these effects, both day-time only and night-time only data were modelled separately. The effect of temperature had a very large evidence ratio above the null, random effects-only model during both the day and night (**Table 5.1B** and **C**). During the day, the model indicated a relative increase in the log-odds of activity of 7.6% for each unit of scaled increasing temperature, but this only resulted in an absolute increase of 1.8% log-odds probability of activity overall, indicating a weak association (**Table 5.2B** and **C**). As there was little evidence to indicate a strong effect of temperature on activity, no threshold (p50 of a logistic curve) could be identified given the current range of temperatures and activity levels.

Table 5.1: Model rankings of binomial generalized linear mixed effects models predicting the probability of activity with respect to temperature and effects of day and night (dn; positive condition is night) on activity levels ranked by AICc model selection methods. Individual ID was included as a random effect. Model group A represents all day and night data pooled, while B and C represent measures of activity and temperature separately between day and night, respectively.

(A)	Model Formula	*k*	Delta AICc	Weight	Deviance
	Temp$ * dn	5	0	0.80	14132.98
	Temp$ + dn	4	2.8	0.20	14137.81
	Temp$	3	228.6	0	14365.55
	dn	3	310.5	0	14447.52
	null	2	553.8	0	14692.76

(B)	Day Only	*k*	Delta AICc	Weight	Deviance
	Temp$	3	0	1	5551.79
	null	2	127.4	0	5681.24

(C)	Night Only	*k*	Delta AICc	Weight	Deviance
	Temp$	3	0	1	8603.65
	null	2	182.4	0	8788.05

$ represents temperature that was centred and scaled around 0.

Table 5.2: Model parameter beta estimates from generalized linear mixed effects models of temperature (scaled and centred, c.Temp) and day and night (dn, positive condition is night) in predicting the probability of activity with individual ID included as a random effect. Models listed here are those with a delta AICc of 0 from **Table 5.1** corresponding to model results A (day and night pooled), B (Day-time only), and C (Night-time only).

(A)			95% Confidence	
Parameter	Estimate	Std. Error	Lower	Upper
(Intercept)	0.288	0.103	0.085	0.491
c.Temp	0.075	0.039	-0.001	0.151
dn	-0.622	0.042	-0.706	-0.539
c.Temp*dn	-0.095	0.043	-0.181	-0.010

(B)			95% Confidence	
Parameter	Estimate	Std. Error	Lower	Upper
(Intercept)	0.294	0.105	0.086	0.502
c.Temp	0.076	0.044	-0.011	0.164

(C)			95% Confidence	
Parameter	Estimate	Std. Error	Lower	Upper
(Intercept)	-0.339	0.103	-0.542	-0.136
c.Temp	-0.038	0.030	-0.097	0.020

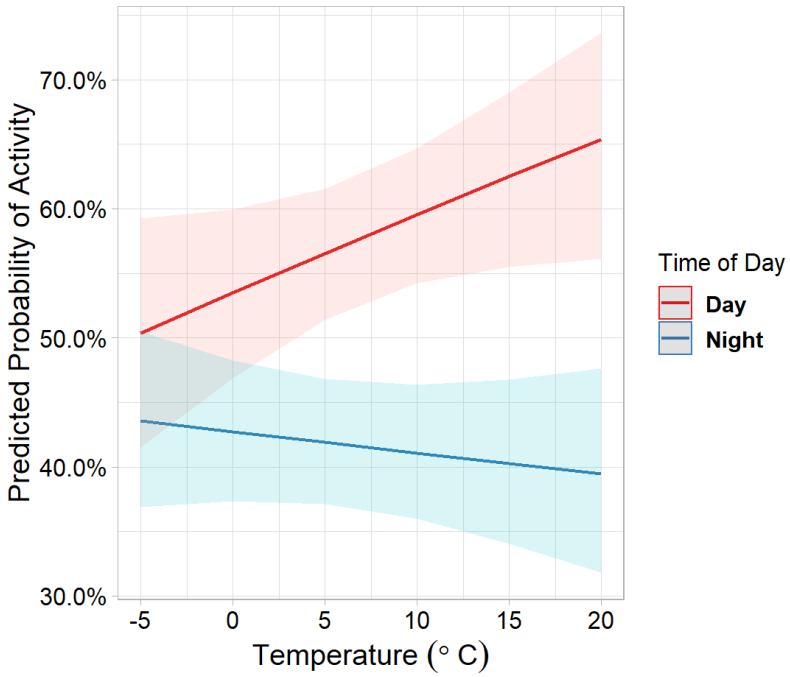


Figure 5.6: Plot of the predicted interaction effects of day and night with temperature on the probability of activity in female lactating grey seals. While there is an apparent increase in activity with warmer temperatures during the day (red line), overlapping confidence intervals indicates this relationship may be weak.

5.3.2 – Energy management strategies in lactating grey seals

Overall, 2,324 15-min segments of heart rate data were analysed across 24 females across both study years. While there was a great deal of variability between individuals in the slope of the relationship between min-f_H against aux-f_H when regressed (**Figure 5.7**), all females with heart rate data displayed a negative slope, indicating female grey seals employ a compensation model of energy management during lactation according to Halsey et al. (2019). While all females displayed a negative slope, six females did not have a slope that was significantly different from zero (see **Table 5.3**).

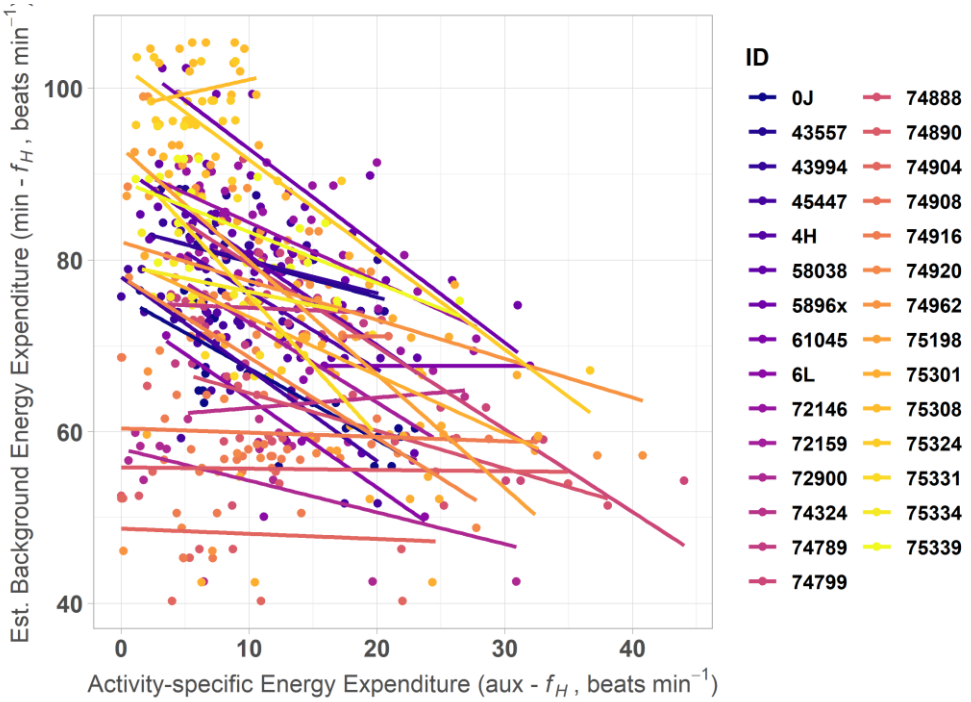


Figure 5.7: Relationship between daily minimum heart rate (min-f_H) and daily auxiliary heart rate (aux-f_H) for each individual lactating female grey seal. Negative slopes indicate that all study females were employing a compensation model approach (fixed, finite resources) to energy management during lactation, however some slopes were not significantly different from zero, as listed in **Table 5.3**.

Table 5.3: Slopes and standard error of individual female energy management modelled as the relationship between min-f_H and aux-f_H from **Figure 5.7**. Individuals with slopes significantly different from zero are highlighted in bold.

ID	Est. Slopes	Std. Err.	*t*-value	*p*-value
0J	**-0.676**	**0.268**	**-2.525**	**0.012**
43994	-0.381	0.294	-1.296	0.196
45447	**-0.656**	**0.317**	**-2.068**	**0.040**
4H	**-0.903**	**0.374**	**-2.416**	**0.016**
58038	-0.357	0.339	-1.052	0.294
5896x	**-1.028**	**0.188**	**-5.480**	**< 0.001**
6L	**-1.312**	**0.322**	**-4.077**	**< 0.001**
72146	**-0.802**	**0.167**	**-4.801**	**< 0.001**
72159	**-1.021**	**0.291**	**-3.506**	**0.001**
72900	**-0.426**	**0.177**	**-2.404**	**0.017**
74789	-0.319	0.310	-1.029	0.304
74799	**-0.962**	**0.126**	**-7.621**	**< 0.001**
74888	**-0.434**	**0.183**	**-2.376**	**0.018**
74904	-0.069	0.210	-0.328	0.743
74916	-0.071	0.157	-0.453	0.651
74920	**-1.050**	**0.387**	**-2.717**	**0.007**
74962	**-0.809**	**0.144**	**-5.637**	**< 0.001**
75301	**-1.115**	**0.147**	**-7.577**	**< 0.001**
75324	**-0.585**	**0.346**	**-1.691**	**0.092**
75334	-0.444	0.405	-1.095	0.275

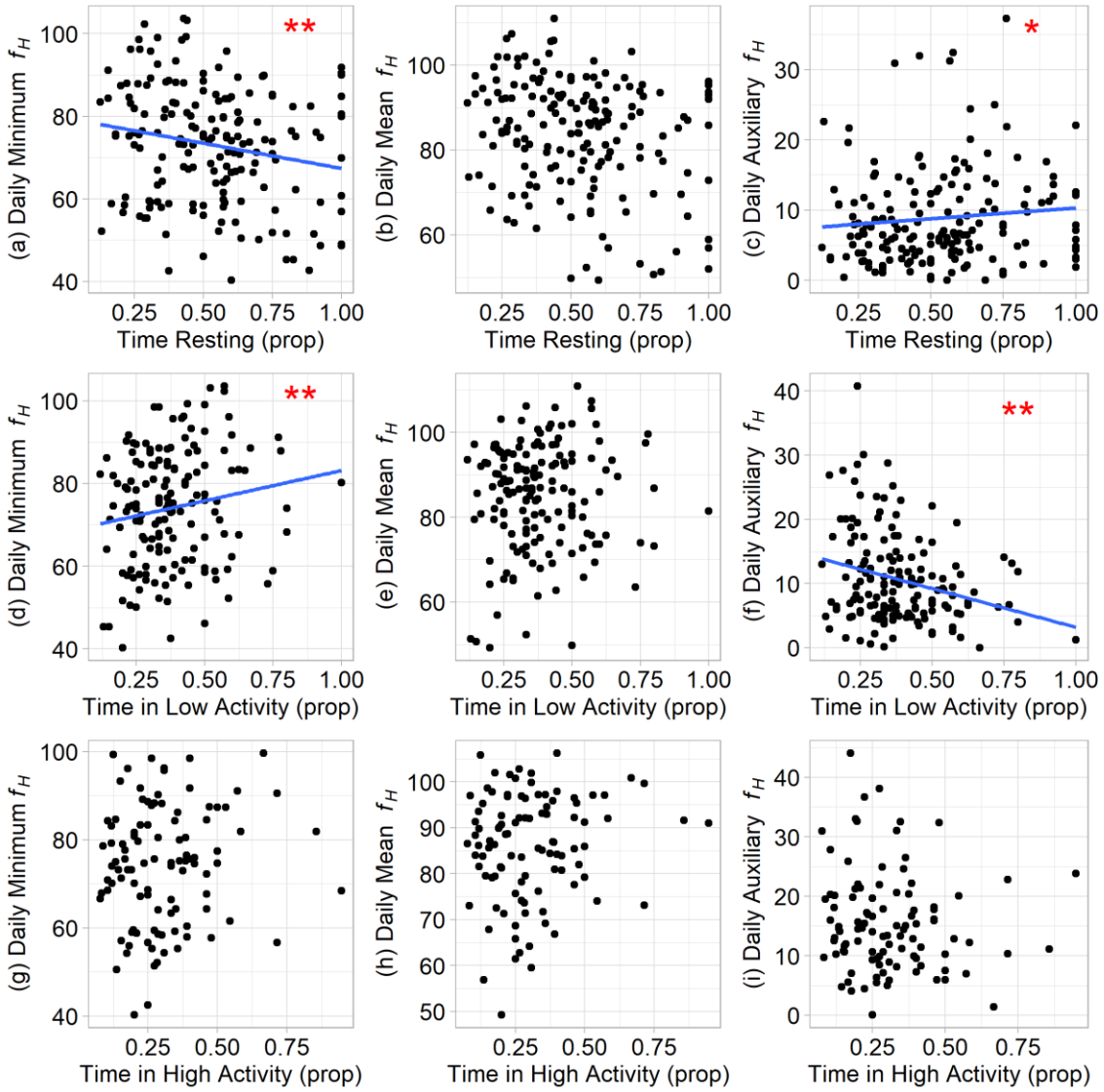


Figure 5.8: Relationships between daily minimum heart rate (min-f_H, beats min^{-1}, a proxy for resting metabolic rate), daily mean-f_H (beats min^{-1}, a proxy for daily energy expenditure), and daily auxiliary heart rate (aux-f_H, beats min^{-1}) to time spent inactive, in a low activity state, and time spent in a high activity state during heart rate collection periods for lactating female grey seals. Time in each activity was determined as the proportion of 15-minute segments of measured heart rate where mean VeDBA was either binned into inactive, low, or high activity states. Significant relationships are noted in red stars.

As expected, a greater proportion of time spent inactive translated to a significantly lower daily resting metabolic rate (min-f_H; β = -11.74 ± 5.4, t = -2.158, p = 0.032; **Figure 5.8a**) as well as a lower yet insignificant daily energy expenditure (mean-f_H; p = 0.385, **Figure 5.8b**), but an unexpectedly significant higher activity-specific energy expenditure (aux-f_H; β = 4.69 ± 2.5, t = -1.821, p = 0.07; **Figure 5.8c**). On the other hand, daily resting metabolic

rate was found to significantly increase with more time spent in low activity (min-f_H β = 19.61 ± 7.9, t = 2.45, p = 0.01; **Figure 5.8d**) as well as a higher yet insignificant increase in daily energy expenditure (mean-f_H p = 0.20; **Figure 5.8e**), while activity-specific energy expenditure decreased significantly (aux-f_H β = -11.35 ± 3.94, t = -2.87, p = 0.004; **Figure 5.8f**). No discernible relationship could be found for time spent in a high activity state in relation to any of the three heart rate-derived energy usage metrics (all p > 0.10; **Figure 5.8g-i**).

Accelerometer-derived energy usage did not scale with daily energy expenditure as derived from heart rate data. There was no relationship (slope not significantly different from zero) between mean VeDBA and mean-f_H, even after the removal of a single outlier with excessive leverage (linear model, β = 0.0002 ± 0.0006, p = 0.673; **Figure 5.9**).

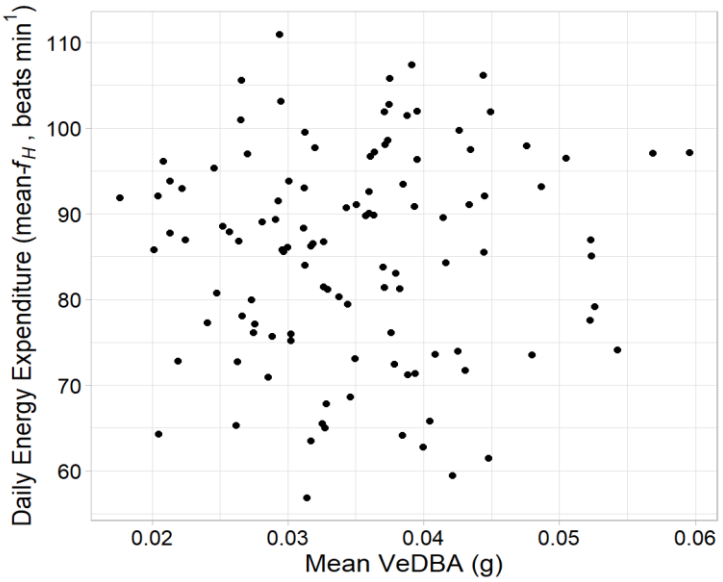


Figure 5.9: Scatter plot of mean activity level (mean VeDBA) and daily energy expenditure (mean-f_H). Blue line characterizes line of best fit from a linear model of mean VeDBA predicting mean-f_H with 95% confidence intervals in grey. There is no meaningful relationship between mean activity level as determined by accelerometry and daily energy expenditure as determined from heart rate methods in lactating female grey seals (β = 0.0002 ± 0.0006, p = 0.673).

Across the island, temperatures experienced by seals when present varied considerably, both spatially (with respect to locations on the colony) and temporally (as indicated by date;

Figure 5.10). Each of the three heart rate-derived energy usage metrics for each day period was regressed against the maximum daily temperature to assess responses of energy partitioning within the compensation model (**Figure 5.11**). Estimated resting metabolic rate (min-f_H) was found to decrease overall as maximum daily temperature increased (β = -0.048 ± 0.01, t = -4.45, p < 0.001). Daily energy expenditure (mean-f_H) was also found to decrease as daily maximum temperatures increased (β = -0.071 ± 0.01, t = -5.95, p < 0.001), but activity-specific energy expenditure (aux-f_H) showed no relationship to the range of maximum temperatures measured (β = -0.019 ± 0.02, t = -0.95, p = 0.341).

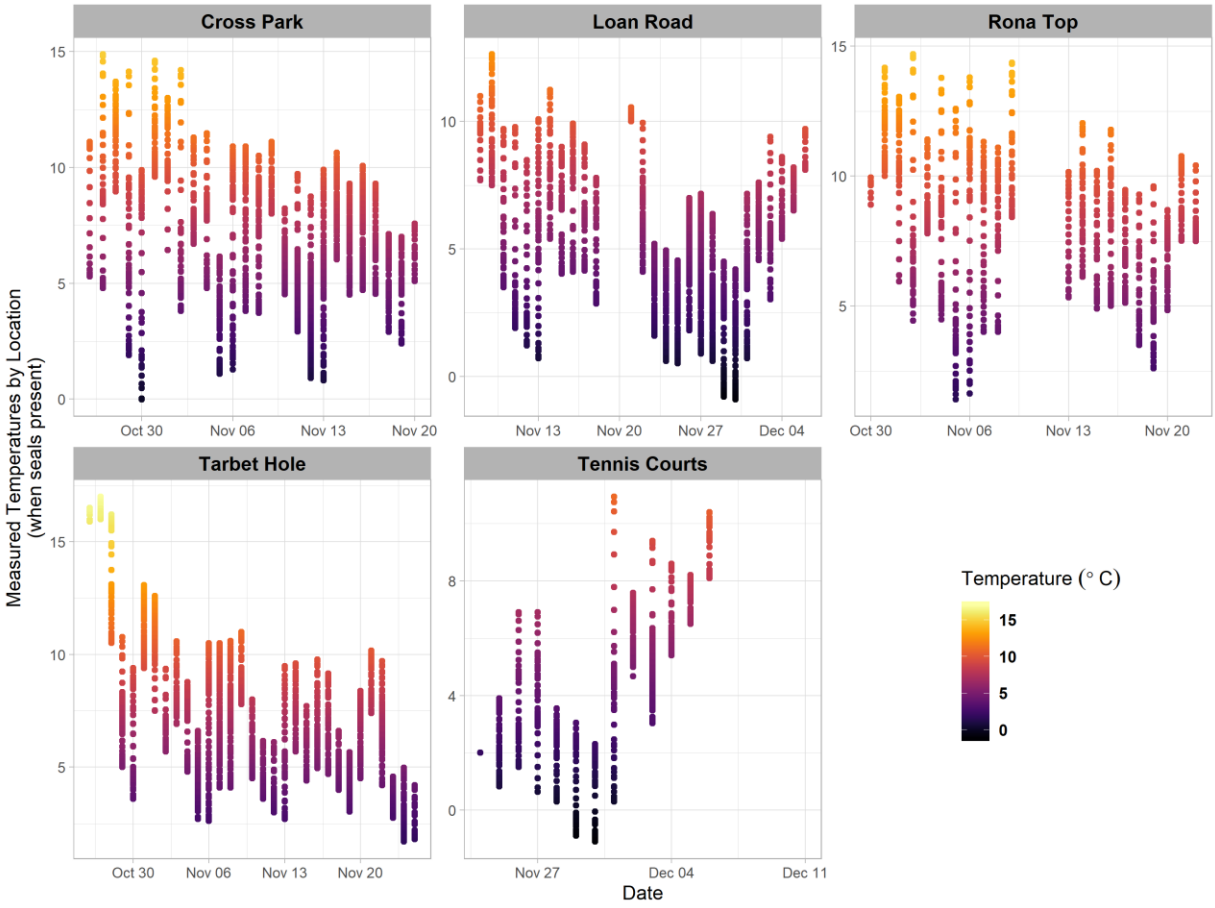


Figure 5.10: Temperature measured by iButton data loggers across 5 of the sub-regions of the Isle of May while study seals were present within the study areas. As there was no iButton within the bounds of the Loan Road, the closest iButton in the centre of the Tennis Court (TC – see **Figure 5.2**) was used as the reference.

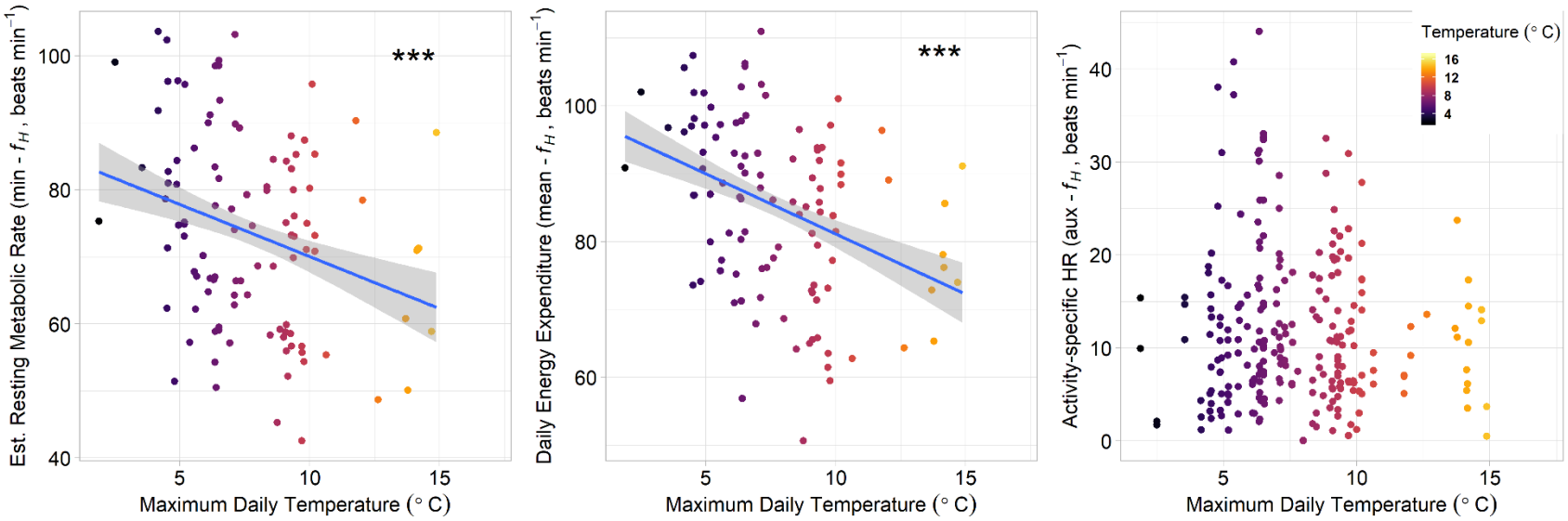


Figure 5.11: Temperature influences energy management. Effect of daily maximum temperature derived from iButton nearest to each study seal on estimated resting metabolic rate (min-f_H, beats min^{-1}), daily energy expenditure (mean-f_H, beats min^{-1}), and activity-specific heart rate (aux-f_H, beats min^{-1}) in lactating grey seals during daylight hours. Blue lines indicate the line of best fit with 95% confidence intervals in grey as determined from a linear model. Significant relationships were found between min-f_H and mean-f_H and daily maximum temperature ('***', $p < 0.001$).

5.3.3 – Stress-coping styles and fitness outcomes

Of the 24 individuals pooled across years, 7 were classified as having low HRV (proactive stress-coping style), while the remaining females were deemed to have high heart rate variability (reactive stress-coping style). Females measured between years were consistently classified as having low or high HRV (n_{recaps} = 6; Cohen's kappa = 1). Time spent inactive and in a low activity state did not differ between individuals with low or high HRV when examined using an Wilcoxon rank sum test (Inactive, W = 57, p = 0.974; Low Activity, W = 56, p = 1), but those with low HRV spent more time in a high activity state than those with high HRV, though the difference was not significant (W = 65, p = 0.578; **Figure 5.12**).

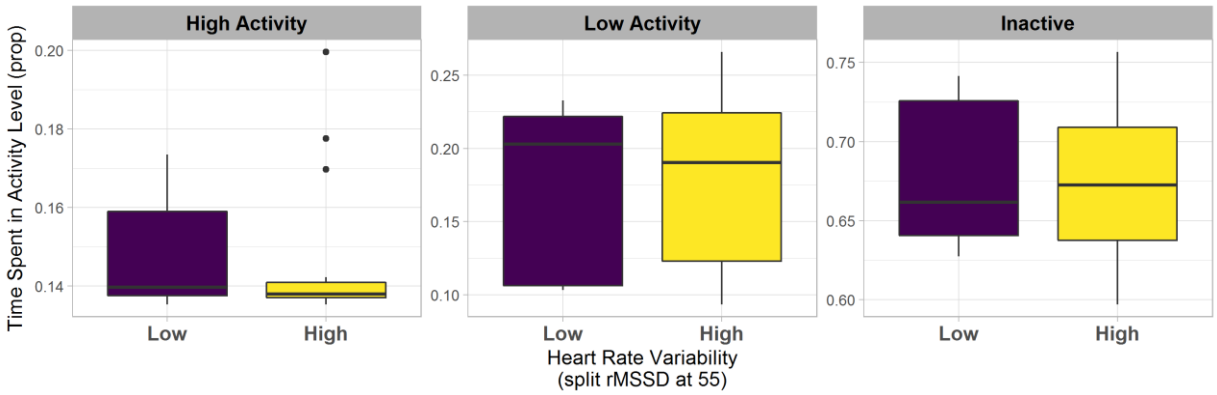


Figure 5.12: Differences time spent in each activity level as a function of heart rate variability in lactating grey seals. There was no significant difference detected between high and low heart rate variability in relation to time spent in each activity level (Wilcoxon rank sum: High Activity, W = 65, p = 0.751; Low Activity, W = 56, p = 1.00; Inactive, W = 57, p = 0.974).

Differences in rMSSD were found to be significant predictors of mean values for all three heart rate metrics (**Figure 5.13**). HRV was negatively associated with mean daily resting metabolic rate (min-f_H; β = -19.553 ± 2.31), whereby individuals with high HRV were found to have significantly lower mean resting metabolic rates (t = 9.319, p < 0.001). Likewise, HRV was negatively associated with mean daily energy expenditure (mean-f_H; β = -17.308 ± 2.51); individuals with high HRV were found to have significantly lower mean daily energy expenditure than those with low HRV (t = 8.541, p < 0.001). The relationship between rMSSD and mean daily activity-specific energy expenditure was less clear, where females with high

HRV were found to only have marginally higher aux-f_H (β = -2.085 ± 0.94, t = -2.061, p = 0.053).

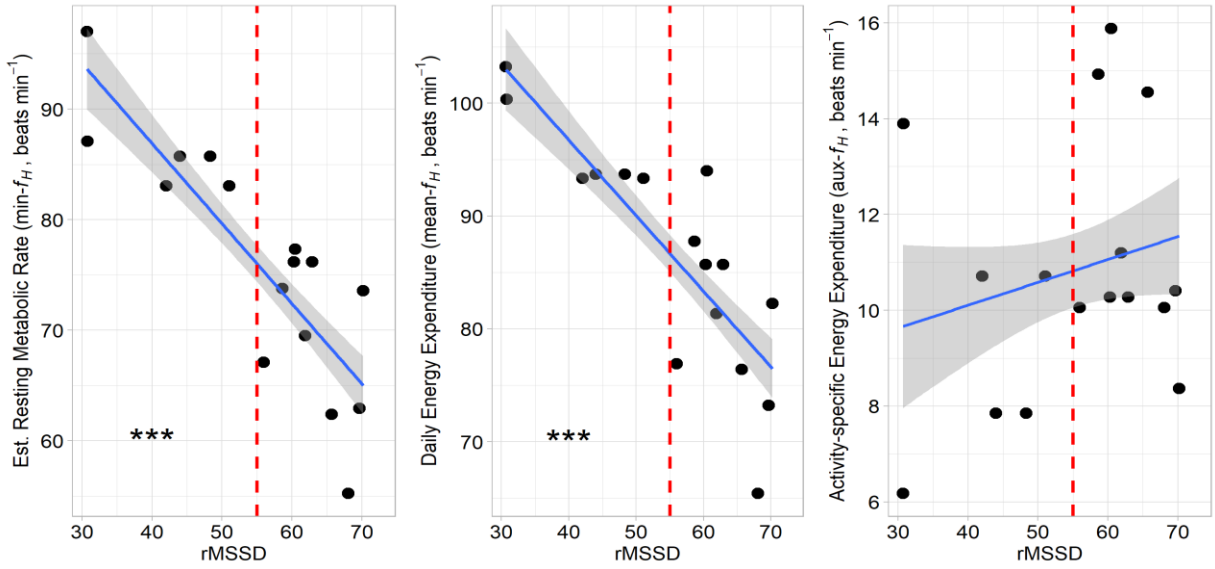


Figure 5.13: Heart rate variability as expressed through the root mean square of successive differences, rMSSD, regressed against mean daily resting metabolic rate (min-f_H), mean daily energy expenditure (mean-f_H), and activity specific energy expenditure (aux-f_H) as indicated by blue lines with grey 95% confidence intervals. Broken red line indicates previously noted inflection point between high and low heart rate variability. Females with high heart rate variability (HRV, above 55 rMSSD) were found to have a significantly lower mean daily min-f_H and mean-f_H than females with low HRV (***, $p < 0.001$). HRV was only marginally significant in its association with mean daily aux-f_H (p = 0.053), indicating a potential trend.

Differences in HRV also accounted for significant differences in female MTeff (t = 2.362, df = 43.836, p = 0.022). Females with low HRV had a higher MT_{eff} (46.7 ± 8.8%) than those with high HRV (41.1 ± 10.2%; **Figure 5.14**), though females with a low HRV were significantly less variable than those with a high HRV when compared to their deviance from the grand mean (t = -2.362, df = 43.836, p = 0.011). HRV also accounted for significant differences in mdml (t = 3.702, df = 65.215, $p < 0.001$). Females with a lower HRV had a significantly greater rate of mass loss (21.36 ± 1.11 g kg^{-1} d^{-1}) over lactation than females with a higher HRV (19.46 ± 3.13 g kg^{-1} d^{-1}), though those females with low HRV were significantly less variable in their deviance from mean mass loss than those with high HRV (t = -3.702, df = 65.215, $p < 0.001$; **Figure 5.15**).

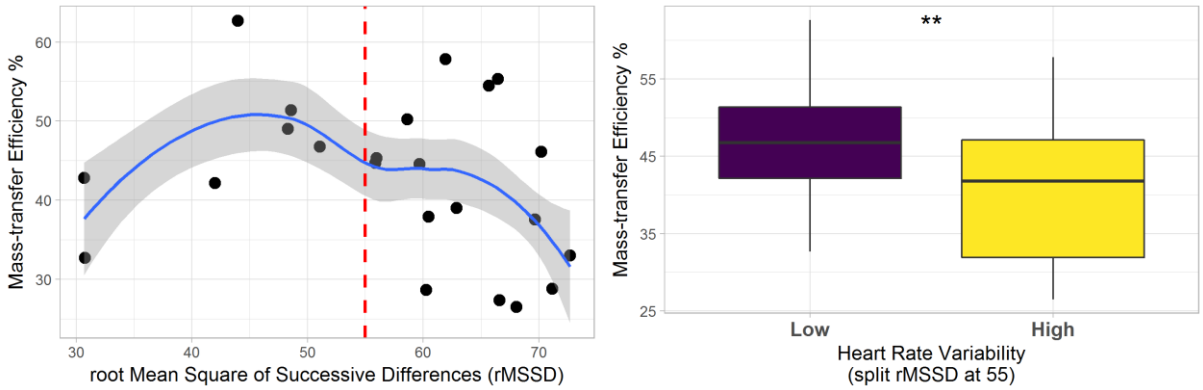
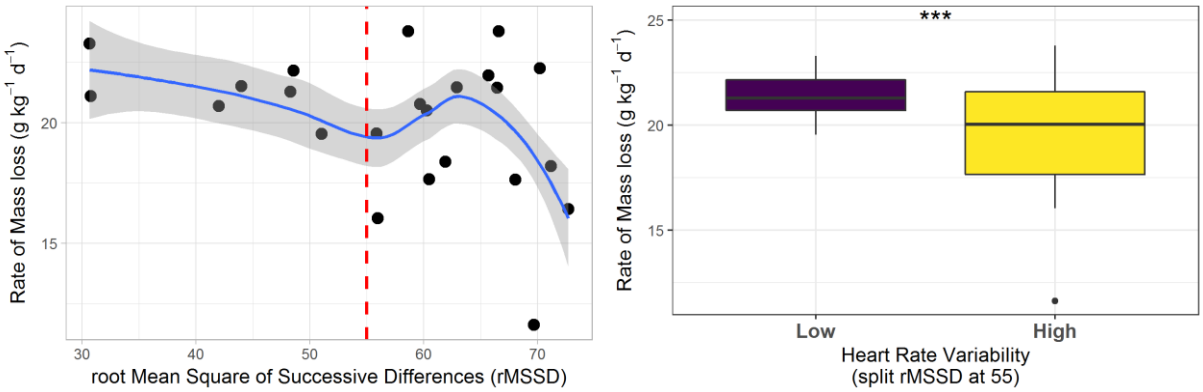


Figure 5.14: Relationship between heart rate variability (as measured through rMSSD) and mass-transfer efficiency in female lactating grey seals. Females with low heart rate variability had significantly higher mass-transfer efficiency than those with high heart rate variability (t = 2.362, df = 43.836, p = 0.022, as indicated by "**"), although females with low heart rate variability were significantly less variable in deviance from the grand mean in mass-transfer efficiency than those with high heart rate variability (t = -2.362, df = 43.836, p = 0.011).

Figure 5.15: Relationship between heart rate variability (as measured through rMSSD) and mass-specific rate of mass loss (g kg^{-1} d^{-1}) in female lactating grey seals. Females with low heart rate variability were found to have significantly higher rates of mass loss (t = 3.702, df = 65.215, p < 0.001), and were much less variable in deviance from the grand mean (t = -3.702, df = 65.215, p < 0.001), than those females with high heart rate variability.



5.4 – Discussion

Grey seals appear to operate in a compensation energy management framework, as indicated by the negative relationship between min-f_H and aux-f_H. They also appear to be remarkably consistent within themselves between years in their energy usage strategies, exhibiting a similar temporal pattern of mean daily activity across lactation between years. Temperature as well as day and night were found to influence the probability of activity over time, though the effect sizes and log-odds of temperature in all models were relatively small. In addition, complementary relationships were found with regards to metabolic rate (min-f_H)

and activity-specific energy expenditure (aux-f_H) and time spent in various levels of activity, but with no significant relationship between activity and daily energy expenditure (mean-f_H). Also, no relationship was found between the measures of accelerometer-derived activity level and the average rate of energy used as determined from heart rate monitors, indicating that accelerometers may not be a valid stand-alone proxy for measuring energy usage for grey seals in this context. When examining the effect of temperature, females were more likely to be active in warmer weather during the day but did not appear to incur a higher cost in terms of activity-specific energy expenditures. Instead, females apparently lowered their estimated resting metabolic rate and subsequently their daily energy expenditure to account for an increasing burden of thermoregulatory costs. While activity levels are likely already minimized, heart rate variability as a measure of stress-coping styles was found to have an influence on overall daily energy expenditure and estimated metabolic rates as well as having an apparent effect on ultimate fitness outcomes of mass transfer and mass loss rates over lactation.

5.4.1 – Energy management strategies in grey seals

As expected, lactating female grey seals in this study were found to show a negative relationship between daily min-f_H and daily aux-f_H (**Figure 5.7**). This negative relationship indicates that as the cost of auxiliary processes increases, females must in turn take away energy allocated to maintenance costs (min-f_H as a proxy for resting metabolic rate), demonstrating that females operate within a compensation model of energy management. As discussed in Careau and Garland Jr. (2012), the compensation model directly quantifies discrete energy trade-offs within a fixed system. This can be illustrated through **Figure 5.16**, scenario A, whereby individuals balance the demands of background processes (min-f_H), such as metabolism and milk energy output, against demands for activity (aux-f_H). The authors define that within this compensation model, any energy demanding activity must be

compensated for by a reduction in another component of the energy budget to maintain a constant, ideal daily energy expenditure (Careau and Garland Jr. 2012). As a result, this model predicts that there should be no discernible relationship between activity levels and daily energy expenditure and that a negative relationship between activity and basal metabolic rate should exist, which was confirmed with the results of this study (**Figure 5.7**). More recent work by Halsey et al. (2019) has demonstrated that these relationships between activity, basal metabolic rate (alternatively defined as background processes), and total daily energy expenditure hold true across a variety of taxa who exhibit this compensation model (also referred to as an allocation model by the authors) of energy management.

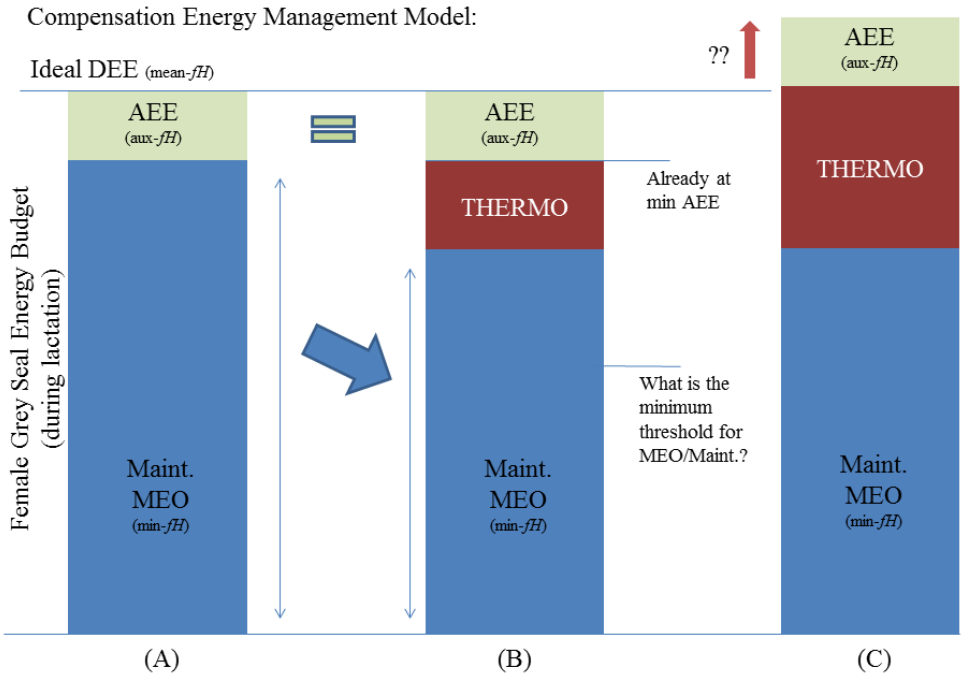


Figure 5.16: Diagram illustrating the compensation energy management model as determined through heart rate methods across three hypothetical cases of daily energy expenditure (DEE; mean-f_H) associated within a hypothetical ideal energy budget. Blue bars indicate the maintenance costs (Maint.; approx.. resting metabolic rate), combined with milk energy outputs (MEO) since it is continuous production throughout lactation relative to time scales considered here and inseparable from maintenance costs within measured minimum heart rate (min-f_H). The green bars indicate what is measured with activity-specific energy expenditure (AEE; aux-f_H). Case A illustrates the likely energy partitioning associated with lactating female grey seals in this study when they are within their thermal neutral zones. Case B shows how females appear to be responding to an increase in the daily maximum temperature as indicated by the red box for physiological thermoregulatory costs in the current study. Case C illustrates what may happen if a theoretical minimum for both maintenance and AEE were reached, but thermoregulatory costs continued to increase and may push females into an independent model of energy management where females exceed their ideal daily energy expenditure.

As predicted by the compensation model, the current study found no significant relationship between mean activity levels and daily energy expenditure (mean-f_H; **Figure 5.8b, e, h**). Significant, contrasting relationships, however, were noted between estimated resting metabolic rates (min-f_H) and activity-specific energy expenditure (aux-f_H) with time spent both inactive and in low activity states (**Figure 5.8a, c, d, f**). More time spent inactive appeared to correlate with lower resting metabolic rate (min-f_H). Conversely, costs associated with auxiliary processes went up as time spent inactive increased. The opposite was found for time spent in low-level activity states. While this could just be a reflection of aux-f_H's negative relationship to min-f_H, it may indicate that state switching to activity after a greater period of time inactive may incur a higher cost than one might expect at first glance. Within a compensation framework, increasing metabolic rate following a period of relative metabolic depression should incur a higher energy cost than decreasing metabolic rate. This idea has been investigated extensively within the hibernation physiology literature (Speakman et al. 1991; Humphries et al. 2003; Heldmaier et al. 2004; Karpovich et al. 2009; Robbins et al. 2012). Periods of arousal from torpor or hibernation states, where metabolism is depressed, were found to have a significant cost on the energy reserves of mammals overwintering (Thomas et al. 1990; Speakman et al. 1991). Evidence in arctic ground squirrels (*Spermophilus parryii*) suggests that it requires a higher rate of oxygen consumption to rewarm following hibernation than to re-cool as the metabolism is depressed back into a torpor state (Karpovich et al. 2009). While internal temperatures were not measured in the current study, the observed metabolic depression and the likely depression of internal temperature associated with days where an individual spends more time inactive suggests that switching out of an extremely low energy state incurs a higher activity-specific cost than spending more time in a low activity state. In this framework, female grey seals studied here who spent more time inactive may be trading-off the benefits of decreased metabolism with the risk of a higher than average activity-

specific energy expenditure associated with the disturbance from inactivity. A lack of a relationship between high activity and heart rate metrics likely reflects the limited amount of time that females tend to spend in high activity behaviours. Significant relationships would likely arise in individuals who spend exceptionally more time in high energy behaviours. Differences in energy usage between different types of high activity behaviours may also show a more relevant relationship (e.g. aggression may incur a higher cost than locomotion). This lack of association between the two is further supported by the non-significant relationship between the rate of activity (mean VeDBA) and daily energy expenditure (mean-f_H; **Figure 5.9**).

Intuitively, female grey seals exploit a compensation model of energy management in order to minimize the negative impacts of such an energetically demanding period. Given that grey seal females are iteroparous, females must find balance between how much energy they are able to expend on their young of that year and how much should remain available to minimize the deficit to be made up during the subsequent moult and gestation periods. Females showed individually different slopes in relation to their compensation model of energy management. In a theoretical sense, these slopes may reflect differences in the hypothetical energy threshold that each female grey seal may be able to constrain themselves to with respect to their metabolic scope (intercept), over the course of lactation. A higher metabolic scope, also defined as a higher maximum metabolic rate (e.g. Auer et al. 2018), would allow females a greater degree of plasticity (steeper slope) in altering their energy usage and maintaining this compensatory relationship. Previous work has demonstrated that female seals may adjust their reproductive output between years when faced with changing environmental conditions prior to birth in order to maximize lifetime reproductive output (Pomeroy et al. 1999; Bowen et al. 2015; McMahon et al. 2016; Desprez et al. 2018; Kalberer et al. 2018). It is unclear to what degree these apparent limits may fluctuate between years or if there is an optimum energy

usage threshold that may be reached at peak productivity in reproduction as female size plateaus at about 10-15 years of age in grey seals (Bowen et al. 2006). A similar pattern in peak productivity was found in the smaller female Antarctic fur seal, but at a much younger age (*Arctocephalus gazella*; (Lunn et al. 1994; Beauplet et al. 2006). While the current investigation was unable to track these energy usage strategies over many years, individual slopes of min-f_H and aux-f_H may relate to the age- and size-specific compensation limits and constraints that each female may exploit from year to year.

Within these apparent compensation limits, one other possibility is that the observed differences in the compensatory relationship (slopes) may reflect changes in milk energy outputs from day to day for an individual. While milk energy outputs tend to change over the course of lactation, it may be assumed that more short-term fluctuations in the rate of production and outputs are likely negligible within a day (Mellish et al. 1999a). However, energetic outputs from milk production are likely contained in what was measured as a proxy basal metabolic rate (min-f_H) during this intensive period of lactation. Individual grey seal enzyme activity is a primary driver of the efficiency of milk energy production over lactation (Mellish et al. 2000). While it is unclear how optimum enzymatic activity may change over the life of an individual, those with a lower min-f_H may be more efficient in milk production through higher enzyme activity resulting in an overall lower energetic cost to milk output.

5.4.2 – Measuring energy usage over time and its limitations

Early research into the theory of animal movement and specific dynamic body acceleration promised the utility of accelerometers as an accurate and remote method for measuring energy expenditure without the use of expensive injectables or metabolic chambers (Wilson et al. 2006; Fahlman et al. 2008; Halsey et al. 2008; Gleiss et al. 2011). As a result, many species-specific calibrations were conducted between activity levels, usually overall

dynamic body acceleration or VeDBA, and rate of oxygen consumption (Wilson et al. 2006; Halsey et al. 2009b, 2011b; Lyons et al. 2013; Volpov et al. 2015b; Grémillet et al. 2018). Following the successful calibration of accelerometry derived activity in a handful of species, many researchers pushed the technology forward for use on field deployments to make comments about the relationship between total activity and absolute energy usage over the same period of time (Gómez Laich et al. 2011; Wright et al. 2014; Maresh et al. 2015; Jeanniard-du-dot et al. 2016, 2017). Many of the methodologies presented in these papers, where summed activity is regressed against summed energy usage, may have fallen into what is known as the 'time-trap': Relationships of energy usage tend to grow with time and may cause spurious correlations if an element of time is included on both sides of a calibration curve (Halsey 2017). Re-testing some of these observed relationships using acceleration as a proxy for energy usage has shown this was likely violated in many studies involving pinnipeds, especially at sea (Ladds et al. 2017a).

In the current study, no meaningful relationship could be found between mean VeDBA and mean-f_H (**Figure 5.9**), further supporting the work by Ladds et al. (2017). This is not to say that accelerometry does not serve a purpose in measuring energy usage over time. While small magnitude behaviours like fidgeting may not incur much of an energetic cost cumulatively, behaviours at the extremes of an individual's capabilities as measured through accelerometers, such as extended exercise like swimming or running (high activity), should scale accordingly with the rate of energy used (Hawkes et al. 2014; Wang et al. 2015; Halsey 2016; Careau 2017). This is especially true for ectothermic animals where the relationships between exercise and energy expenditure can easily be calibrated relative to the external temperature following Q10 scaling (Udyawer et al. 2017). Heart rate, while generally assumed to be more accurate than accelerometry at determining energy usage over time, can still be prone to error if the relationship is not properly calibrated to a species (Butler et al. 2004; Portugal et al. 2016).

Many of these relationships are also confounded by the physiology of the individual. Individual differences in blood oxygen stores, stroke volume, muscle fibre type, and species-specific adaptations to exercise, such as diving, may mean that relationships calibrated on only a few individuals may perform poorly when applied to others (Horning and Trillmich 1997; King et al. 2004; Noren et al. 2005; Hindle et al. 2011; Horning 2012). A few studies have calibrated oxygen consumption rates for several age classes and sexes of grey seals while diving and on land (Boily and Lavigne 1995, 1996, 1997). However, no study to date has measured these relationships in pregnant or lactating female grey seals, limiting the conclusions that can be made about absolute energy usage in the current study. One study using injectables found that background metabolic compensation in lactating female grey seals was well over seven times what would be predicted by Kleiber's mass-specific metabolic rate equations (Kleiber 1961; Mellish et al. 2000). Once a calibration curve could be set for lactating females with regards to accelerometry and heart rate methods, one may easily be able to scale what was measured here into actual, discrete energy usage and partitioning over the course of the breeding cycle.

5.4.3 – Stress-coping styles and energy trade-offs

While no link was found between intensity of activity and stress-coping styles, females classed as proactive were found to have an overall higher mass-transfer efficiency, but at a greater cost of mass loss per day (**Figures 5.14** and **5.15**). Underlying this, significant relationships were noted across stress-coping styles between estimated resting metabolism and daily energy expenditure (**Figure 5.13**); individuals classed as proactive were found to have overall higher resting metabolism and higher daily energy expenditures than reactive females. This result contributes to a growing body of evidence linking consistent individual differences in stress coping styles to underlying physiology. Consistent differences in neurochemical response pathways and heart rate variability in relation to stress have been linked to differences

in behaviour, dominance rankings, and underlying metabolism in a variety of taxa (Careau et al. 2008; Careau and Garland Jr. 2012; Briefer et al. 2015; Portugal et al. 2016; Careau 2017). Koolhaas et al. (2011) defined stress as those conditions of the environment that include unpredictability or uncontrollability that may exceed the natural capacity of an organism to regulate. Several important factors can be measured to judge how an individual responds to these acute stressors: The initial magnitude of the response to a stressor and the subsequent time of recovery to some baseline level (Koolhaas et al. 2011). While some authors describe stress reactivity and stress-coping styles as perpendicular axes of stable trait characteristics, the two likely represent the short term responses to stress and the compounding effects of how stress is dealt with and anticipated over time, respectively (Koolhaas et al. 2010). Within lactating grey seals, consistent individual differences in stress reactivity have already been linked to fitness outcomes (Twiss et al. 2012a). This has been further supported by the results presented here showing that compounding effects of stress management, as expressed through rMSSD, have a direct influence on energy management and energy usage (**Figure 5.13**).

Consistent differences in stress-coping styles have shown well established links to life history traits as an explanatory variable in predicting and quantifying differences between species along a fast-slow life-history continuum (Jones et al. 2008; Careau et al. 2009; Debecker et al. 2016). Grey seals, like most large carnivores, exhibit a slow life-history. Females produce a single, large offspring in each breeding cycle after a delayed age-at-first reproduction, with the additional flexibility of multiple breeding cycles throughout an individual's life (Pomeroy et al. 1999; Bielby et al. 2007). Within a species, consistent individual differences in activity, metabolism, and personality are linked to, and often predictive of, individual fitness outcomes as defined by the pace-of-life syndrome hypothesis, an extended concept of the fast-slow life-history continuum to include the effects of physiology (Réale et al. 2010; Le Galliard et al. 2013; Závorka et al. 2015; Binder et al. 2016; Debecker et

al. 2016; Guenther 2018). More specifically, the proactive-reactive spectrum of behaviours (measured previously in Twiss et al. 2012) and physiology (measured here as differences in HRV determined through rMSSD) seem to support the hypothesis that these pro-reactive traits can allow for vastly different life-history strategies and trade-offs within a species (Réale et al. 2010). Differences in boldness, one of the behavioural traits associated with these pro-reactive stress-coping styles, have correlated to metabolic capacity in fish (measured as the difference between basal metabolic rate and maximum metabolic rate; Binder et al. 2016). Given the physiological underpinnings already found for stress-coping styles and stress reactivity in this chapter and in previous work (e.g. Koolhaas et al. 1999), the relationship between min-f_H and aux-f_H within an individual may indicate consistent individual differences in a physiological reaction norm, namely an individual's metabolic scope (intercept) and plasticity capacity (slope; Dingemanse et al. 2010).

Within a compensation model, Careau et al. (2008) alternately describe proactive individuals as potentially requiring a lower basal metabolic rate in order to afford the high energetic demands of the suites of behaviours (high activity-specific energy expenditure) associated with a proactive lifestyle as an alternative means for allocation of energy. Personality differences in metabolic rate are largely driven by overall differences in behaviour relating to net energy gain or expenditure, especially with respect to stress responses, but are context dependent upon the energy management strategy employed in the system (e.g. Mathot et al. 2019). For example, individuals with a proactive coping style who often display more dominance, boldness, and foraging intensity should, and often do, have a higher metabolic rate in order to execute behaviours that result in energetic gains in the long run (Réale et al. 2010; Mathot et al. 2019). However, under a compensation model of energy management, individuals displaying these same behavioural traits should actually reduce their metabolic overheads in order to accommodate the higher activity costs that these traits are associated with

(Careau et al. 2008; Réale et al. 2010). Most of the female grey seals in this study that exhibited this proactive lifestyle were found to have consistently higher resting metabolism, daily energy expenditure, and mass-transfer efficiency (MT_{eff}) than those of the reactive lifestyles, but at a greater cost to the individual (mdml) and apparent increase in the mean resting metabolic rate (min-f_H). While this appears to contradict the alternate allocation model presented by Careau et al. (2008), min-f_H as a measure of resting metabolic rate would be unable to separate out basal metabolic rate from other background processes. This suggests that proactive females may still be experiencing a lower 'true' basal metabolic rate, but that an increased min-f_H may actually be capturing an overall higher energy throughput into other background processes such as milk production energy and may explain in part consistently higher mass-transfer efficiencies and daily mass loss rates for proactive females.

Females exhibiting a reactive lifestyle were found to be more variable, but sometimes with a higher than average, in mass-transfer efficiency and rate of mass loss than proactive individuals. Reactive individuals, as a consequence of their behavioural traits and underlying physiology, are often observed to be more sensitive to environmental cues than the more aggressive, proactive individuals, which may be reflected in the slight increase in activity-specific energy expenditure among individuals with high HRV (**Figure 5.13**; Careau et al. 2009; Réale et al. 2010; Debecker et al. 2016). Given the right environmental, density- or resource-dependent circumstances, these reactive females would likely outperform their proactive counterparts. While beyond the scope of this thesis, lifetime reproductive output as a consequence may be equivalent among individuals with vastly different pace-of-life syndromes, especially considering the negative effects on longevity that have been associated with a faster, proactive lifestyle within a population (Nunn et al. 2003; Jones et al. 2008; Réale et al. 2010). In addition, pace-of-life syndromes within mothers likely plays a large role, not only in the survival of their offspring, but also with regards to that offspring's personality

development and subsequent knock-on effects of individual differences (Monestier et al. 2015). Tracking these consequences and trade-offs in stress-coping style may shed further light on pace-of-life syndromes in other capital breeders.

5.4.4 – Thermal considerations for energy management

Maximum daily temperature played a significant role in predicting resting metabolism and daily energy expenditure, but not activity-specific energy expenditure in grey seals. This appears to indicate that females will lower these two aspects of energy management in response to an apparent increase in thermal burden (**Figure 5.11**). The relationships suggest that the energetic costs of activity (aux-f_H) did not respond to increasing temperature measured in this study, but instead females must decrease their resting metabolic rate (min-f_H) and daily energy expenditures (mean-f_H) in order to allow for thermoregulatory costs to keep within their own compensation strategy constraint window (scenarios A and B, **Figure 5.16**); only minimal evidence was detected to suggest that females are behaviourally thermoregulating (**Figure 5.6**). In theory, there should be a minimum threshold to which a female grey seal can reduce her resting metabolic rate and milk energy outputs in order to successfully complete lactation. Activity-specific energy expenditure is already apparently minimized for most individuals in the current sample population, regardless of stress-coping style (**Figure 5.12**). Given that ambient temperatures measured here are within the acceptable range for a grey seal's predicted mass-specific thermal neutral zone, it may be that the reason for early abandonment is due to the extra burden of thermoregulatory costs (2016, n = 4; 2017, n = 4; **Table 2.1**), pushing some females beyond the limits of their fixed energy stores. In this case, physiological requirements dictated from the environment may exceed availability. This potential relationship can best be described in scenario C of **Figure 5.16**. Grey seals are already operating well above their normal range of mass-specific metabolic rates during lactation (Mellish et al. 2000), so even a small increase in temperature, even within their projected thermal neutral zone, may be outside

their physiological tolerance range and metabolic capacity (Boily and Lavigne 1995; Mellish et al. 2000). The relationship between the probability of activity and temperature was not strong enough to determine a specific threshold (**Figure 5.6**, **Table 5.2**), though females as a whole were more active in 2016 than 2017 (**Figure 5.3**), likely as a result of differing thermal regimes between seasons (comparing **Figure 5.10** and **Figure 2.5**). As a consequence of using loggers, the data that were retrieved are likely biased towards those who have not been pushed past their theoretical tipping point. Tagging best practices also means study animals were chosen that were in the best condition and are the most stable, excluding those at either end of the spectrum of the compensation energy usage framework. If a theoretical minimum was reached for both maintenance and activity costs, an increasing burden of thermoregulatory costs may force individuals into an independent energy model where daily energy expenditure continues to increase irrespective of basal maintenance processes (Careau and Garland Jr. 2012). An extended time spent within an independent energy usage model may push some females into an extreme energy debt that may result in skipping the following breeding season (Pomeroy et al. 1999), or may be unrecoverable.

Species can exhibit a variety of responses to increased thermal burdens in a changing climate. On a population level, many species have shifted their ranges to occupy more suitable spatial habitat or shifted phenology of key life history events (Hill et al. 2003; Boveng et al. 2008; Bowen et al. 2017). Most seals within the subfamily Phocinae (true seals in the Northern Hemisphere) are thought to have evolved such a contracted lactation in order to breed on ephemeral ice floes (Fedak and Anderson 1982). Grey seals at the current study colony supposedly time the breeding cycle to exploit seasonal temperatures well within their thermal neutral zone while on land (Twiss et al. 2002; Stewart et al. 2014). However, as the climate is projected to warm (Jenkins et al. 2008), phenological and geographical shifts in the timing and location of breeding may be needed to exploit a more ideal temperature range wherever

possible, as has already been noted in the west Atlantic population of grey seals (Bowen et al. 2017). Several species, both terrestrial and aquatic, already exhibit differences in activity through behavioural thermoregulation, by exploiting optimum temperatures both within fine spatial and temporal scales in order to minimize thermal costs to an individual (Campagna and Le Boeuf 1988; Hill et al. 2003; Wolf et al. 2005; Beentjes 2006; Fossette et al. 2012). Seals on the Isle of May are limited in their ability to behaviourally thermoregulate. If not located near a pool of water, females may have to leave their pups for an extended period of time to access freshwater to drink and to cool off, but at the cost of precious energy reserves and leaving their pups unattended and open to harassment, potentially preventing reunification (Redman et al. 2001; Stewart et al. 2014). When analysing the probability of activity, a 10% increase in activity was noted during the day at the maximum temperature experienced by seals, or less than a 1% increase in activity per unit of scaled temperature (**5.3.1**). While it is unclear how much of an increase in activity would cause appreciable changes in energy usage without some kind of oxygen consumption calibration curve, it does indicate that females may be moving more as temperature increases. While some argue that the temperatures measured in this study are well within an individual's thermoneutral zone, this may not reflect the state of an individual's core temperature, nor the thermal radiance experienced on such an exposed colony.

5.4.5 – Conclusions and forward directions

The way forward, it seems, is to combine the use of accelerometers with other sensors and physiological measures, such as heart rate, to make sure that the relationships between proxies and actual energy used scale appropriately and to uncover more meaningful relationships to activity levels and energy management over time (Bishop et al. 2015c). Individual stress-coping style in relation to energy usage and management appear to indicate that lactating female grey seals exhibit a variety of pace-of-life syndromes. In the current

study, examining accelerometer-derived activity bins combined with aspects of heart rate variability revealed that proactive individuals appear to be exploiting a strategy of higher energy throughput into pups, but at a greater cost to the self without spending more time active. Several significant relationships between apparent energy usage as determined through heart rate measures and local temperature were also found that impact on a grey seal's compensation energy management. Individuals seem capable of minimizing background processes in order to compensate for an increasing burden of thermoregulatory costs. However, as with relationships between activity levels and absolute energy usage, only those cases towards the extremes of an individual's tolerance would likely show a significant relationship. Lactating female grey seals remain inactive most of the time for a reason. Individuals are limited by their own specific energetic constraints and must remain within that apparent ceiling if a successful lactation is to take place. Only a longitudinal study following the development and survival of offspring and the life-time reproductive output of the female can elucidate the long-term consequences of different life-history energy usage strategies and their ultimate utility for unpacking and predicting population dynamics.

Chapter 6:

Can you feel my heart beat? Assessing the use of externally-mounted accelerometers to detect resting heart rate, breathing rate and apnea in a free-ranging lactating pinniped

Manuscripts from this Chapter:

SHUERT, C. R., P. P. POMEROY, AND S. D. TWISS (*in prep*) Apnea on land saves energy and conserves water: Using externally-mounted accelerometers to detect resting breaths during lactation in a free-ranging phocid seal, with applications to other terrestrial vertebrates. [*Author Contributions*: CRS collected the data and performed the analyses with support from SDT and PPP. CRS wrote the paper with input from all co-authors]

6.0 – Abstract

Pinnipeds are lauded as the champion athletes of the mammalian dive reflex. While holding their breath (apnea), seals are observed to undergo extreme bradycardia associated with periods of diving. These dive reflex characteristics relating to heart rate and concomitant breathing are often retained, even when pinnipeds are on land for extended periods of time. While the mechanisms driving the degree and frequency to which seals will hold their breath on land are still debated, tracking these parameters has been previously unattainable without direct observation. This chapter sought to use accelerometers as a novel method for detecting heart rate, individual breaths, and apnea on land in female grey seals. Peaks in filtered data from back-mounted accelerometers were compared to heart rate monitor-derived R-peaks or to visually-derived breath events to determine detection accuracy for both heart rate and breathing rate, respectively, while at rest. Individual breaths were detected well with high accuracy. Accelerometry-derived apnea periods were also accurately matched to video footage. On average, females spent 24.8% of resting periods in a state of apnea. Various hypotheses about the function of apnea were also investigated. Spending more time in apnea during lactation appeared to confer significant energy savings both in relation to background metabolism as well as energy expenditure, but apnea also seemed to confer significant water conservation benefits as ambient temperatures rose. Measures of hematocrit over lactation were also significantly related to time spent in apnea, suggesting a link to endogenous aerobic capacity and water conservation. Heart rate, however, was not well detected due to resampling errors; a higher sampling rate for externally-mounted accelerometers may perform better. The methods presented here for detecting breathing rate will be incredibly useful in other pinniped species already equipped with accelerometers, but can also be extended to other terrestrial species.

6.1 – Introduction

6.1.1 – Heart rate, seismocardiography, and respiration

The heart is a complex organ that is modulated by both sympathetic and parasympathetic inputs that control both the timing of heart muscle contractions as well as the overall cardiac output (Schmidt-Nielsen 1997). In healthy individuals, these contractions follow a highly predictable set of electrical impulses, collectively known as the sinus rhythm, that act as a landmark for the various contractions of the heart muscles and subsequent ejection of blood into the body. In the mammalian heart, blood is ejected from the large, muscular left ventricle and passes out into the body through the aortic arch and beyond (Schmidt-Nielsen 1997). This ejection represents the peak of electrical activity and vibration, referred to as the R-peak within the QRS complex of electrical potential, and is used as a landmark for not only measuring the functionality of the heart, but also can indicate the relative changes in cardiac output (amplitude) and heart rate variability (period; e.g. Lu et al. 2009). Following the invention of the first stethoscope in the mid-18th century, monitoring the sounds of the heart, and later heart rate, became an important way to assess and monitor the health and metabolism of an individual that was not only minimally invasive, but also allows for the accurate diagnosis of a variety of conditions within the body. Since this relatively simple invention, ways in which doctors and researchers are able to track and study the heart have expanded dramatically. More advanced techniques have allowed for the detection of electrical activity through the use of electrocardiography (ECG or EKG) as well as more specific heart sounds associated with morphological functioning through echocardiography via ultrasonography (e.g. Peters et al. 2004). While likely the most accurate methods, these two ways of monitoring the heart are fairly expensive and bulky when using hospital-grade diagnostic equipment, often requiring the patient to lay perfectly still while attached to a number of leads.

Advances in microprocessor and sensor technology have allowed for the development of wearable devices to track heart rate. Many are simplified ECG units that measure electrical potential between two leads, worn as either a patch or belt, often capable of transmitting or storing a raw ECG waveform, preferred for detecting problems with electrical output and heart functioning, or returning a simplified R-peak detection output that may be better suited for measuring heart rate and its variability over time (Andrews et al. 1997; Marchant-Forde et al. 2004; Adnane et al. 2009; Williams et al. 2017). Measuring pulse rate as a proxy for heart rate through the means of near-infrared light has also become a common practice and can be especially useful in field situations, but requires placement in an area of the body where blood vessels run close to the surface (Lu et al. 2009; Gil et al. 2010; Lee et al. 2013; McKnight et al. 2019). Most recently, heart rate and heart rate variability have been reliably detected using small motion sensors such as accelerometers (Phan et al. 2008; Pandia et al. 2010; Inan et al. 2014; Bicen et al. 2018). The force of arterial blood should scale accordingly with cardiac output, a product of both the stroke volume and the heart rate, resulting in varying degrees of force and displacement within the body according to vessel size (Schmidt-Nielsen 1997). Seismocardiography specifically measures heart rate associated with reverberations of the heart's contractions against the chest wall using bi- or tri-axial accelerometers sampling at a high rate, while ballistocardiography attempts to capture these same heart vibrations resulting from the whole-body displacement due to the forces associated with the heart pumping and detected through the substrate upon which an individual is situated (Castiglioni et al. 2007; González-Landaeta et al. 2007; Inan et al. 2014). Gyroscopes have also been used in a similar set-up to seismocardiography to reliably extract heart rate as a change in angular displacement of the chest over time (Jafari Tadi et al. 2017). While most of these methods will likely be contaminated with noise when measuring heart rate during periods of movement, they

represent promising developments for measuring resting heart rate and its variability during a variety of clinical scenarios.

The ability to remotely measure respiration rates has also been a focus of development for use in monitoring patient health in biomedical applications. In addition to tracking ventilation in relation to blood oxygen saturation, used a method for quickly identifying problems with lung function, measuring respiration rates overnight can also be used as an indication of sleep apnea and other clinical issues. Respiration rates in a laboratory setting are typically measured through a belt or strap with stretch receptors, but have also been easily identified through the same accelerometer set-ups that measure ballisto- and seismocardiography (Phan et al. 2008; Pandia et al. 2012; Sweeney et al. 2013). By filtering the signal to include only low frequency oscillations, many wearable devices can now sample chest movement associated with breathing rates when an individual is at rest. While the ability to measure respiration rates through a single wearable motion sensor is promising, the ability to detect respiration during activity using this method is still subject to noise and signal contamination from movement. At present, detecting respiration rates while an individual is at rest is likely to be the only reliable period of time to obtain accurate data.

6.1.2 – Measuring heart rate and respiration in wild animals

Tracking heart rate in wild animals has been shown to be a reliable indicator of metabolic capacity, personality differences, stress management, and a variety of other health metrics that make it a very valuable aspect of physiology to capture in wild contexts (e.g. Mohr et al. 2002; Butler et al. 2004; Halsey et al. 2008; Liu et al. 2014; Portugal et al. 2016). While most sensor development with respect to tracking heart rate has been for humans and clinical contexts, there have been several attempts to develop approaches for monitoring heart rate in wild animals. Externally-mounted heart rate monitors with electrode leads, usually measuring

electrical potential and subsequent inter-beat intervals, have been used extensively in terrestrial and aquatic animals in a wide range of sizes and configurations (MacArthur et al. 1979; Gessaman 1980; Laming et al. 1982; Gallivan et al. 1986; Mohr et al. 2002; Green et al. 2005; von Borell et al. 2007; Green 2011; Turbill et al. 2011; Grandi and Ishida 2015; Portugal et al. 2016; Twiss et al. *in review*). Externally attached heart rate monitors have also been used to monitor embryo development in eggs (Pearson et al. 1998; Akiyama et al. 1999; Aubert et al. 2004; Shaffer et al. 2014). While these have been extremely successful in captive contexts or over short-term deployments, wild animals run the risk of becoming entangled in vegetation if the lead wires become loose or risk damaging the unit and the data are often heavily contaminated with motion artefacts from the contractions of the muscle and skin around the lead location. This also becomes a big problem for animals that require streamlined body forms for optimum functionality, such as birds and marine mammals, as the extra drag burden that these tags add may be undesirable and could be potentially deleterious if they exceed recommended tag-body mass ratios (Le Maho et al. 2011; Chivers et al. 2016; van der Hoop et al. 2018).

Several studies have attempted to use partial implants for short-term studies of heart rate where the electrical leads are implanted subcutaneously to overcome some of these motion artefacts (Steiger et al. 2009; Sapir et al. 2010, 2011; Dechmann et al. 2011; O'Mara et al. 2017). Partially-external tags, however, can sometimes have adverse effects on study subjects leading to behavioural changes such as increased preening near the implant and attachment sites in birds, and risks infection (e.g. Enstipp et al. 2015). Fully implantable heart rate monitors have been attempted in a variety of species, but still have varying levels of success across taxa (Woakes et al. 1995; Green et al. 2001, 2009a; Halsey et al. 2010; Chaise et al. 2017). These implantable devices can either be placed subcutaneously (e.g. Chaise et al. 2017), within the body cavity (e.g. Gremillet et al. 2005), or temporarily within the rumen of

ungulates using endoscopy (e.g. Signer et al. 2010). As with subcutaneous leads, the risks associated with surgical procedures means that the need for implantable devices must be heavily justified and vetted with respect to the balancing ethical considerations, device-to-body mass ratios, and study goals (Guillemette et al. 2007; Casper 2009; Portugal et al. 2009; Chaise et al. 2017; Horning et al. 2017). Deployment times and data retrieval success rates are quite variable across all monitor configurations: Some implantable device studies only collected data for a few days while others can last for a few weeks or months, but were all limited to relatively larger bodied species which could cope with the increased mass burden of these devices, even when tag size is reduced (Evans et al. 2013; Chaise et al. 2017). Accelerometers are already a popular sensor included in a variety of tag configurations due to their versatility and, if used alone, present a very small footprint for external mounting. Given the relative success of seismocardiography in humans and ruminants (e.g. Signer et al. 2010), assessing the utility of accelerometers for detecting heart rate in other wild contexts may be a useful way to overcome the various challenges faced with heart rate tags; most accelerometers are very small and may present a minimally invasive technique for determining resting heart rate.

Monitoring breathing rates in free ranging wild animals, however, has seen limited effort. Typically, respiration rates for wild animals are counted visually. In clinical settings, veterinarians and technicians typically count the number of breaths either directly from the nostrils or by monitoring the inflations of an anaesthesia bag (e.g. Walker et al. 2011). In the wild, however, this becomes nearly impossible to measure in free-ranging animals. Respiration rates in marine mammals, and other obligate air-breathing marine animals such as sea turtles, are typically inferred by the number of surfacing events or by direct observation while following focal animals (Würsig et al. 1984; Fahlman et al. 2016; Roos et al. 2016). More often than not, studies where marine mammals are deployed with time-depth recorders use surfacing events as a proxy for respiration events (Würsig et al. 1984; Goldbogen et al. 2008;

Roos et al. 2016). These studies make assumptions about the relationship between surfacing rates and respiration events and often extend this to a measure of metabolic rates, but this proxy can be misleading and regularly over-estimates the relationship with energy usage and oxygen consumption rates (Fahlman et al. 2016). In order to overcome this, several studies have attempted to measure respiration events during surfacing events in marine mammals through the use of acoustic tags (e.g. Fletcher et al. 1996; van der Hoop et al. 2017). While simply listening for breaths may be more straightforward, it is unclear however, if any study has attempted to simply measure the contractions of the chest through the use of animal-borne accelerometers.

6.1.3 – Apnea, eupnea, and pinniped breathing rates

A number of studies over the past few decades have attempted to characterize the heart and breathing rates of marine mammals. Pinnipeds, specifically, are lauded as the champion athletes of the mammalian dive reflex. First demonstrated with forced submersions of seals in the 1940s (Irving et al. 1941; Grinnell et al. 1942; Scholander et al. 1942), seals and other diving mammals are observed to undergo extreme bradycardia in response to dives and apnea. The degree to which these dive responses are expressed are a function of a multitude of factors, including the ontogeny and development of blood and body oxygen stores, water temperature, anticipated dive time and depth, exercise duration, and even disturbance events (Williams et al. 1991, 2015, 2017; Andrews et al. 1997; Burns 1999; Hindle et al. 2011; Kaczmarek et al. 2018). The culmination of these factors represents an individual's aerobic dive limit (Noren et al. 2005; Davis and Weihs 2007; Hindle et al. 2011; Horning 2012; Shero et al. 2015), whereby the sum total of these body oxygen stores and aerobic capacity give an upper limit to the amount of time an individual can stay within the bounds of an aerobic metabolism. These characteristics of the diving adaptations are often retained, even when pinnipeds are on land for extended periods of time. This has been noted in an individual expressing a bimodality of heart

rate, alternating between levels of high (tachycardia) and low heart rate (bradycardia; e.g. Castellini et al. 1986, 1994; Williams et al. 1991; Castellini 1996; Kaczmarek et al. 2018). Concomitant with heart rate, pinnipeds also alternate between periods of regular breathing and breath hold, termed eupnea and apnea, respectively, as would be observed with a normal bout of surfacing and diving (Castellini et al. 1986, 1994; Knopper and Boily 2000; Horning 2012). While the mechanisms driving the degree and frequency to which pinnipeds will hold their breath (and changes in heart rate) on land are still debated (Castellini et al. 1986, 1994; Castellini 1996; Andrews et al. 1997), tracking both these parameters over time can be an important measure of an animal's health and aerobic capacity.

This chapter sought to evaluate if resting heart rate and breathing rate could be extracted and estimated from opportunistically deployed accelerometers. Over the course of a breeding season, 24 adult female grey seals were equipped with a single tri-axial accelerometer on the back, just forward of the shoulder blades, for the duration of their on-land lactation period. Concurrent periods of heart rate data from heart rate monitors were extracted while each seal was upright and at rest and used to compare to concurrent acceleration data. A custom designed peak detection algorithm was used on each segment of band-pass filtered acceleration data to attempt to extract peaks in blood displacement associated with the R-peaks of heart beats and movement of blood through the aortic arch. Heart rate monitor data and accelerometry-derived heart rate were then compared over 15-min segments to see if a reliable measure of mean heart rate could be derived as well as comparable measures of heart rate variability. In addition, peaks in low-pass filtered accelerometry data were compared to visually-derived breath events while at rest to see if accelerometers could detect resting breathing events as well as reliably extract periods of apnea. Detected breaths were compared to concurrent heart rate data to assess its utility in determining periods of apnea and eupnea against periods of bradycardia and tachycardia cycles. It has also been hypothesized that apnea

on land may serve as either an energy saving mechanism or as an adaptation for water conservation in phocid seals (Castellini et al. 1986; Castellini 1996; Andrews et al. 1997). As a result, this thesis also attempted to elucidate the function of apnea by examining individual time spent in apnea in relation to daily ambient temperature, variation in haematocrit, as well as fitness and energy use proxies as derived from **Chapter 5**.

6.2 – Methods

6.2.1 – Heart rate and accelerometry data in situ

For the 2017 field season, 24 lactating female grey seals were equipped with heart rate monitors (HRM) and torso-mounted accelerometers (ACC) as described in section **2.3** in the general methods. The HRM unit consisted of a battery, on board processor, and transmitter attached to two leads extending down the flanks of each study female. Electrode pads were treated prior to deployment with a bleach solution to create a continuous silver chloride surface to detect electrical activity, aided by the application of medical-grade electrogel within the mounting structure against the clipped surface of the pelage. As a function of the tag design, the HRMs only transmit data when in range of a portable base station and do not log data. As a result, only a subsample of heart rate data was available across the length of deployment during lactation. HRMs return corrected R-peak times following removal of artefacts, providing the subsequent inter-beat intervals (IBI) within each trace period, sampling at resolution of 1000 Hz (see Twiss et al. *in review*; **2.3**). ACCs were placed along the dorsal midline anterior to the shoulders so that forward movement of the body was in line with the x-axis of movement, while up and down were captured by the z-axis of movement and were programmed to sample at 50 Hz ± 4 *g* with 8-bits of resolution.

The ability to detect heart rate from ACCs was compared by matching 15-min traces of accelerometry data to concurrent 15-min traces of inter-beat intervals (inverse of heart rate)

collected from HRMs while the seal was at rest. In order to match high quality traces of HRM data with ACC segments, periods of ACC data with overlapping HRM data were only considered if the animal was effectively unmoving (resting; < 0.0275 mean Vectorial Dynamic Body Acceleration, see **2.6-11**) and upright for the majority of the trace (static acceleration signal, see **2.6-1-3**, bounded by positive z-axis values and y-axis between -0.5 and 0.5). ACC segments were time-matched to HRM segments to the nearest second in time. Usable HRM segments were considered to have less than 50% flats and stairs contained within the signal as per Twiss et al. (*in review*) and Brannan (2017). This led to the inclusion of n = 548 15-min segments of overlapping ACC and HRM data. For a large analysis of breathing rate, all 15-min sections of heart rate and accelerometer data where the animal was static, as above, were included in the breathing rate analysis (n = 548).

6.2.2 – Comparison of ACC and HRM heart rates

In order to detect heart rate from ACC data, the raw acceleration signal in the z-axis and y-axis were added together and then filtered using a sharp band-pass finite impulse response (FIR) filter between 5 and 25 Hz in order to capture the estimated variability in individual heart beats for each 15-minute segment. A separate sharp low-pass FIR filter was subtracted from this band-passed signal in order to remove noise from breathing and other low-frequency movements as per Signer et al. (2010). The signal was then enveloped by using an adaptive Hilbert FIR over 25 samples (0.5 s). Peaks were then detected using the 'findpeaks' function in MATLAB (version R2015b), with a minimum peak height of 0.02 and blanking time 0.35 s, corresponding to a heart rate of 170 beats min^{-1} to limit any potential bias detecting true beats and false detections (**Figure 6.1**). This peak detection algorithm returns time locations and height of peaks supposedly corresponding to inter-beat intervals. Any inter-beat interval corresponding to a heart rate greater than 120 beats min^{-1} was removed as this was outside the observed heart rate from the HRM data and likely represented noise of movement. The time

lag (*ms*) between the remaining peaks was first smoothed over a 4 sample window and was then used to calculate instantaneous heart rate (beats min^{-1}). A simple square box filter, modified from Signer et al. (2010), was used to filter the dataset to further remove any outliers over a 15-sample interval and a vertical tolerance of 15, removing only 1% of the total points remaining. After two stages of filtering, instantaneous heart rates were recalculated and compared to the HRM data (e.g. **Figure 6.2**).

This analysis was precluded from directly comparing peak times and inter-beat intervals as the HRM data sampled heart rate at a resolution of an order of magnitude higher than the ACC data. As a result, both the mean and median of heart rate were compared between the ACC (ACC-f_H) and HRM (HRM-f_H) data over each 15-min segment. The agreement of these two measures was assessed using Bland-Altman analyses (Bland and Altman 1986; Marchant-Forde et al. 2004; González-Landaeta et al. 2007).

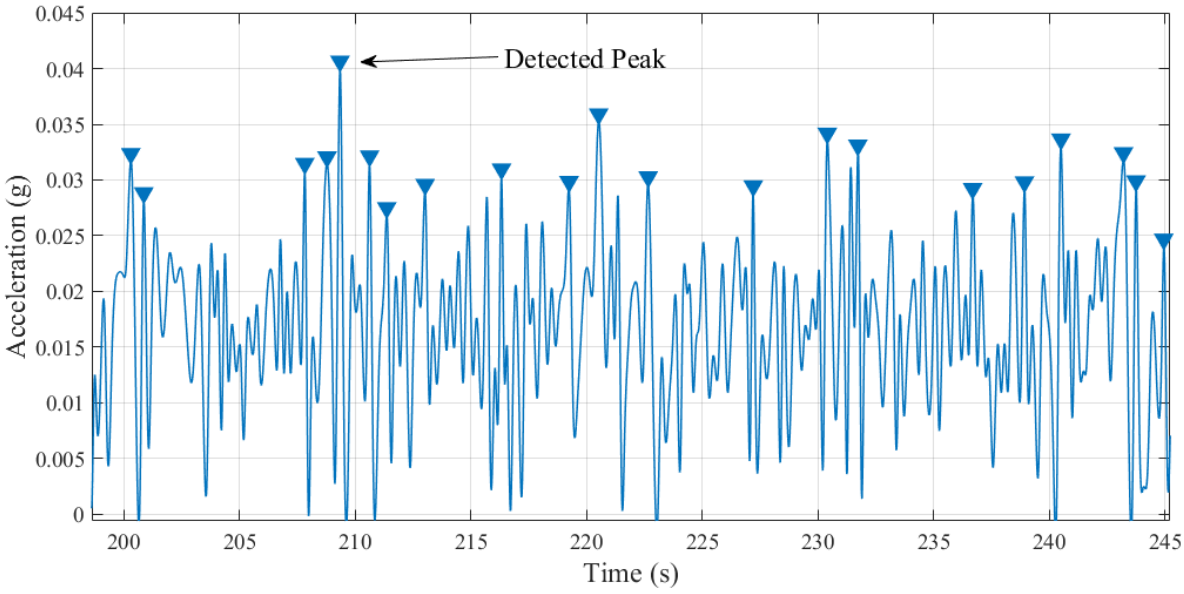


Figure 6.1: Example of the upper envelope of Hilbert-transformed accelerometry data showing the original peaks detected as 'beats' (blue triangles) using the 'findpeaks' function.

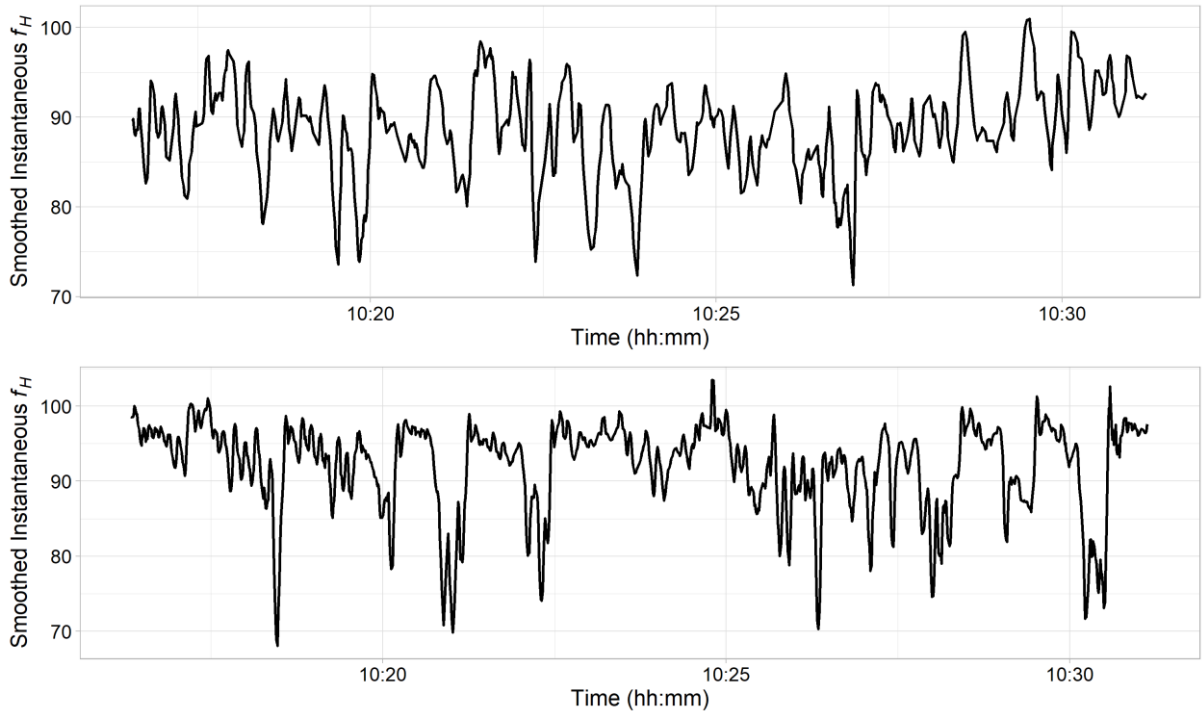


Figure 6.2: Filtered dataset of instantaneous heart rate from ACC data (f_H, beats min^{-1}, top) compared to corresponding heart rate from HRM data (f_H, beats min^{-1}, bottom) over a 15-min trace (time depicted as hh:mm).

6.2.3 – Derivation of respiration waveform and estimated breathing rates

While on land, grey seals typically begin a respiration cycle with a sharp expiration followed by a subsequent inhalation and breathe hold in between cycles. Anterior-posterior (x-axis) movement was thought to contain the most consistent signal of these respiration cycles as chest expansion and contraction of the diaphragm tended to result in a forward displacement of the accelerometer. In order to capture this pattern of movement associated with the respiration waveform, dynamic acceleration in the x-axis (see **2.6-1**) was filtered by executing a sharp low-pass FIR filter (1 Hz) to amplify signals below 60 breaths per minute and contain the oscillating movement of respiration cycles associated with the sharp breath hold at the end of an inspiration. Peaks were detected using the 'findpeaks' algorithm in MATLAB with a minimum peak prominence (absolute amplitude) of 0.01 and a blanking time of 2.5 *s*, specified as the minimum inter-peak distance (**Figure 6.3**). Peaks supposedly corresponding to respiration cycles were labelled by their height and location in time.

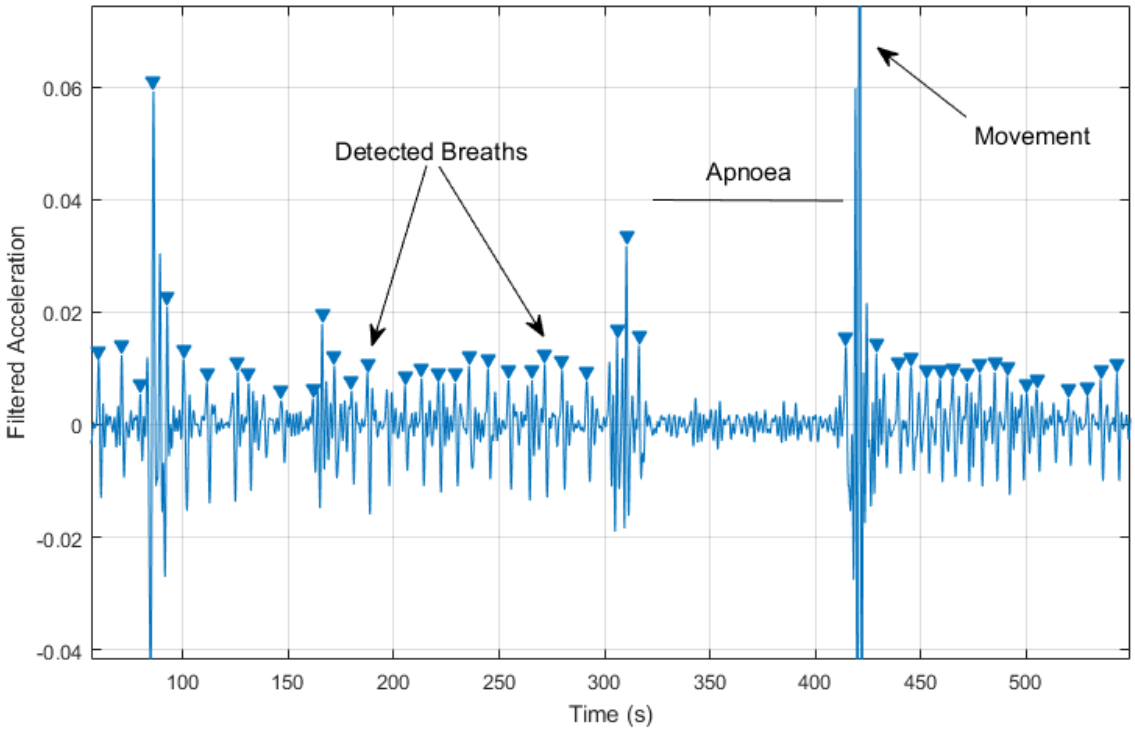


Figure 6.3: Example of breathing waveform as determined through accelerometers (*g*) indicating the peaks detected (blue triangles) through the detect peaks function in MATLAB. A period of apnea is also indicated by the lack of detected breaths. Each peak had a minimum peak prominence of 0.01 *g* and a 2.5 *s* blanking time (acceptable interval between peaks).

A subsample of these breathing rate segments were visually decoded by counting breath events on accompanying video footage. Visually-decoded breath events were time matched to accelerometry-derived breath events. Individual respiration peaks were considered to be accurate if they occurred within 3 s between visual observations and ACC data. The breath detection algorithm performance was assessed by calculating the true positive rate of detection (TPR), also known as recall (see **Chapter 3**, **Eq. 3-5**) and positive predictive value (PPV), also known as precision (see **Eq. 3-4**). Breath event detection was also assessed by deriving the miss rate and false discovery rate (FDR) of the detection algorithm to the visually decoded breaths, calculated as;

$$Miss\ Rate = \frac{FN}{TP + FN} \quad \textbf{(Eq. 6-1)}$$

$$False\ Discovery\ Rate\ (FDR) = \frac{FP}{TP + FP} \quad \textbf{(Eq. 6-2)}$$

As previously stated, values closer to 1 for all event detection metrics represent better algorithm performance. The removal of peaks greater than 0.1 *g* was also examined as a simple method for improving detector fit. All data were compared to visually decoded breaths as well as visually decoded breath and movement periods to assess the source of any detector miss classifications. Once the accuracy of this peak detection algorithm was verified, absence of detected breaths across the duration of deployment were assessed against visually decoded breath hold events to determine if ACC methods were a reliable indicator of periods of apnea. Apnea was defined as periods with more than 11 seconds between breath cycles (or inter-breath intervals) for both ACC and visually-decoded data. The agreement between estimates of apnea from accelerometers to those obtained from video footage was assessed through Bland-Altman analysis (Bland and Altman 1986).

6.2.4 – Physiological drivers of apnea

The percent time spent in apnea from ACC data was investigated as a function of individual. In order to elucidate the possible reasons for apnea on land, the proportion of time spent resting in apnea was investigated in relation to several potential driving variables that may indicate its function as a water conservation mechanism, an energy conservation strategy, as a result of the metabolic consequences of stress-coping styles, or an indication of inherent diving ability (Castellini et al. 1986, 1994; Castellini 1996; Andrews et al. 1997; Careau et al. 2010; Kaczmarek et al. 2018). As a way to investigate the link between apnea and water conservation, the percentage of time spent in apnea over a day as measured through accelerometers was regressed against local daily maximum temperature across the island from iButtons deployed around the island (see **2.7**). Increasing time spent in apnea in relation to warmer temperatures may aid in water conservation by reducing the amount of time expiring and losing moisture to the air. Various indices of energy allocation were also compared to daily mean time in apnea in order to uncover any evidence of energy saving mechanisms

associated with apnea, including daily heart rate metrics related to resting metabolism (min-f_H) and daily energy expenditure (mean-f_H; see **5.2.2**); any negative relationship between energy usage and time spent in apnea should point towards this being an energy savings mechanism. The mean time spent in apnea over the duration of lactation was also compared for each female to estimated rates of mass loss (g kg^{-1} day^{-1}) and mass-transfer efficiencies (**2.6**) to evaluate if overall patterns in time spent apneic have any observable effects with short-term fitness outcomes.

During the 2017 season, blood samples were taken at both the initial and final handling events in order to get measures of haematocrit from packed cell volume. Blood was taken from the epidural sinus shortly following initial restraint following darting using a spinal needle and EDTA or Li-Heparinized vacutainers. Whole blood samples were spun in triplicate using a microcapillary centrifuge for 5 minutes before measuring the packed cell volume (%PCV). The remaining whole blood was then centrifuged for 15-min to archive the remaining plasma. While including a measure of haemoglobin would certainly give a better idea as to diving ability, the change in haematocrit between final and initial capture events was investigated in relation to the maximum time spent in apnea. Haematocrit fluctuates regularly, increasing drastically during breath hold events as an adaptation to diving, even while on land (Castellini et al. 1986; Horning and Trillmich 1997; Noren et al. 2005; Greig et al. 2010; Hindle et al. 2011), and also with relative hydration. As a result, one may expect that an animal that is able to spend more time in apnea may be able to increase baseline haematocrit over time more effectively or may reflect internal hydration state.

6.3 – Results

6.3.1 – Detection of heart rate

A total of 548 15-min segments of concurrent heart rate and accelerometry were analysed across 24 female grey seals. The filtering and peak detection algorithm presented here was unable to reliably detect resting mean heart rate across the majority of 15-min traces. Bland-Altman analysis indicated that on a whole, mean ACC-f_H over estimated mean HRM-f_H by 1.69 beats min^{-1}, but with extremely wide confidence limits of greater than ± 30 beats min^{-1} (**Figure 6.4**; Bland and Altman 1986). Early season ACC-f_H detections performed slightly better than late season (mean difference of 0.03 and -2.43, respectively), but again suffered from extremely wide confidence limits of greater than ± 30 beats min^{-1}. ACC-f_H was best detected for binned HRM-f_H in the range of 75 to 85 beats min^{-1} and 85 to 95 beats min^{-1} for both mean and median values of heart rate, but overall did not provide consistently accurate estimates of heart rate. Full Bland-Altman statistics can be found in **Table 6.1**.

Table 6.1: Bland-Altman statistics from comparisons of measuring mean heart rate (beats min^{-1}) from accelerometers (ACC-f_H) against those measured from a heart rate monitor (HRM-f_H). Samples of heart rate segments (n_{seg}) for the full dataset, as well as comparing detectability between detections from the first half (early) and second half (late) of lactation period across individuals to model differences in body condition. In order to decompose the detectability of heart rate, segments were binned into ranges of heart rate (10 beats min^{-1}) from HRMs and compared.

Comparison		n_{seg}	mean HR			median HR		
			Mean Diff.	Lower Limit	Upper Limit	Mean Diff.	Lower Limit	Upper Limit
Full		*548*	-1.69	-34.46	31.08	-37.48	-2.66	32.17
Early		*206*	0.03	-32.42	32.47	-37.25	-1.62	34.02
Late		*328*	-2.43	-35.70	30.83	-37.83	-3.03	31.77
Binned HR (beats min^{-1})	< 55	*40*	-34.56	-40.90	-28.22	-41.33	-36.13	-30.93
	55 - 65	*45*	-24.43	-35.76	-13.10	-38.81	-29.05	-19.29
	65 - 75	*69*	-16.13	-25.08	-7.17	-33.31	-19.41	-5.50
	75 - 85	*123*	-3.72	-14.02	6.58	-15.89	-3.05	9.80
	85 - 95	*147*	7.34	-5.94	20.63	-5.51	7.28	20.08
	> 95	*124*	16.50	3.80	29.19	3.32	15.64	27.97

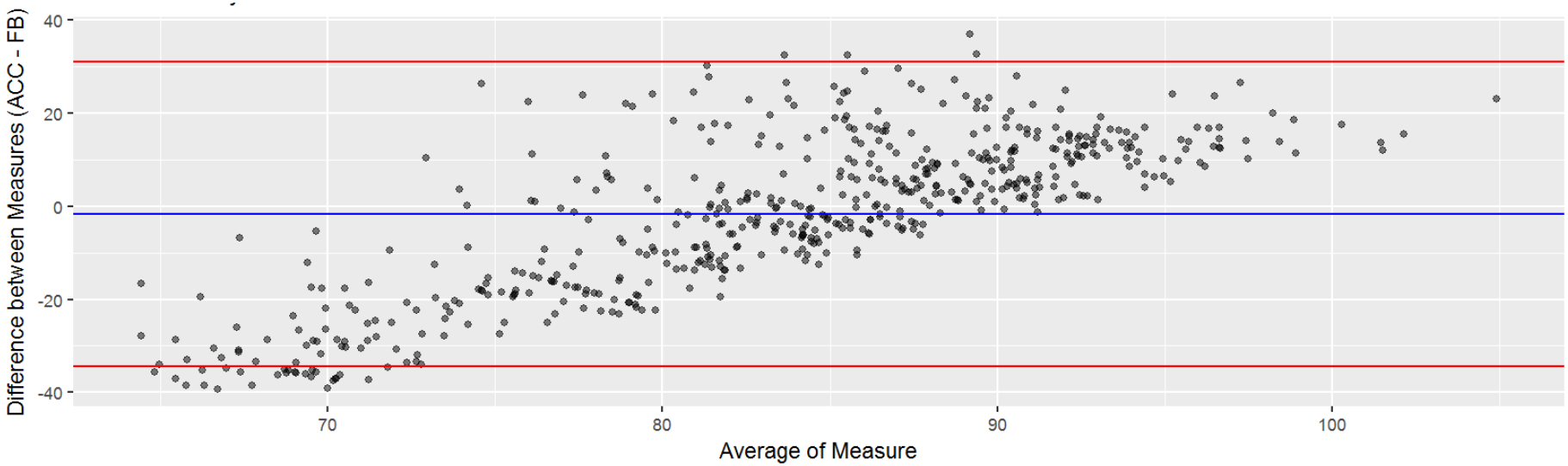


Figure 6.4: Bland-Altman plot of agreement of measures of mean heart rate (difference between measures, beats min^{-1}) between ACC and HRM data. Blue line shows the mean difference between the two measures, while the red lines indicate the upper and lower 95% confidence ranges. Bland-Altman statistics given in **Table 6.1** for all comparisons.

6.3.2 – Detection of breathing rates

A total of 109 15-min segments of video corresponding to concurrent sections of ACC derived breaths were visually-decoded to count individual breath events and test the accuracy of the breath detection algorithm (n_{ind} = 18). This video footage constituted just over 20% of all ACC segments used, totalling over 30 hours of footage and approximately 14,000 individual breaths. Data from several ACC tags appeared to exhibit significant, but non-linear clock drift relative to the computer data corresponding to HRM-f_H segments and needed to be manually adjusted. Once drift was accounted for in visual comparisons, the detector algorithm accurately flagged individual breaths within the ACC data (**Figure 6.5**). Across 15-min segments of ACC and HR data, individual breaths were correctly identified at a rate of 0.785 ± 0.138 (TPR), missing on average 21.4% of real breaths (Miss Rate; see **Table 6.2**). However, the detector algorithm regularly overestimated the number of individual breaths at a rate of between 24.9-64.1% across the interquartile range (FDR; **Table 6.2** and **Figure 6.6**). Differences in detection between data including periods of movement indicated that 5.6% of false positive peaks were due to visually-detected periods of movement (FDR; see 'Breaths only' vs. 'Breaths and Move.' **Table 6.2**). Excluding acceleration peaks greater than 0.1 only decreased the number of false positives by about 2% (FPR), indicating that the majority of false peaks were due to minor movements and would be inseparable from true positive peaks of breathing rates in blind applications of accelerometers (e.g. ACC < 0.1; see 'Filtered -' data comparisons **Table 6.2**). Additionally, a high degree of individual variability in the rate of detection for all performance metrics was noted (**Figure 6.7**).

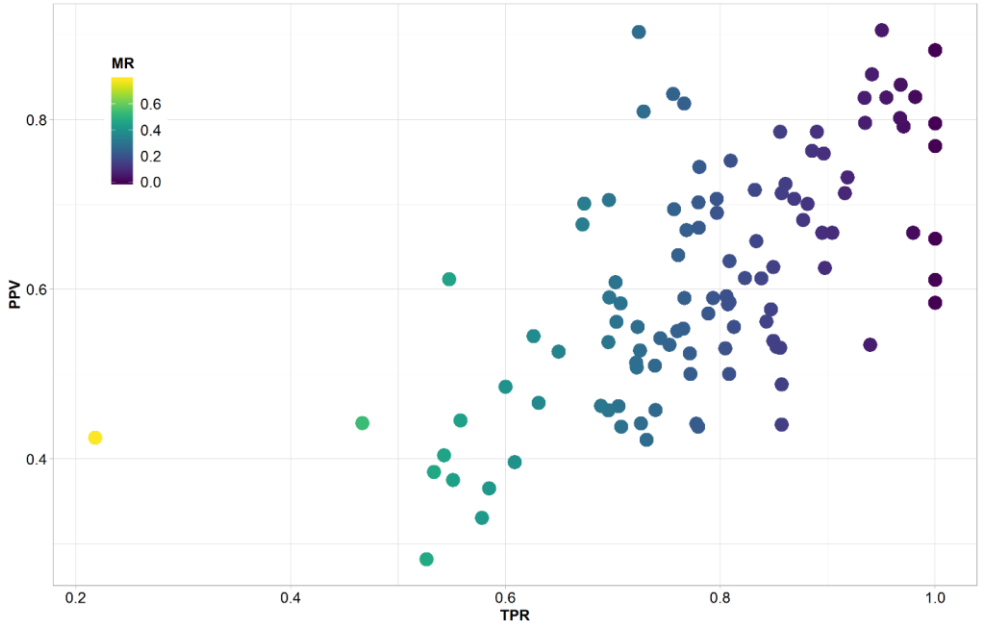


Figure 6.5: Overall breathing rate detector algorithm performance across 15-min segments of resting (n = 109) as indicated by the scatter of true positive rate (TPR), positive predictive value (PPV) and miss rate (MR). False discovery rate (FDR) is not included here as it is the opposite of PPV. Values closer to 1 indicate better model performance.

Table 6.2: Event detection algorithm performance statistics between breaths detected through ACC against visually decoded video footage, described by true positive rate (TPR), positive predictive value (PPV), miss rate, and false discovery rate (FDR). Algorithm performance is compared against visually decoded data including breaths ('Breaths only') as well as including periods of movement ('Breaths and Move.'). In an attempt to remove exclude movement, the ACC-derived breaths were filtered to exclude peaks greater than 0.1 and compared to detecting only breaths ('Filtered – B. only') and breaths and movement ('Filtered – B. & M.').

	Metric	Mean	Std. Dev.	IQR
Breaths only	TPR	0.792	0.125	0.153
	PPV	0.559	0.147	0.222
	Miss Rate	0.207	0.125	0.153
	FDR	0.440	0.147	0.222
Breaths and Move.	TPR	0.710	0.120	0.143
	PPV	0.559	0.147	0.222
	Miss Rate	0.289	0.120	0.143
	FDR	0.440	0.147	0.222
Filtered – B. only	TPR	0.764	0.129	0.148
	PPV	0.573	0.146	0.211
	Miss Rate	0.235	0.129	0.148
	FDR	0.426	0.146	0.211
Filtered- B. & M.	TPR	0.685	0.122	0.149
	PPV	0.573	0.146	0.211
	Miss Rate	0.314	0.122	0.149
	FDR	0.426	0.146	0.211

Figure 6.6: Example comparisons of detected breathing events from the ACC data (small red ticks) as compared to video decoded breaths (small blue ticks) against heart rate (red line; beats min^{-1}) from HRM over a 15-min period for three different types of 'resting' traces (Event Time, hh:mm). Panel (a) represents an animal at rest with fairly regular breathing patterns with regularly oscillating heart rate with a brief period of apnea. Panel (b) shows regular oscillations between high heart rate coinciding with eupnea and low heart rate coinciding with apnea with relatively low variability and sharp transitions between. Panel (c) indicates regular cycles of apnea and eupnea, but with regular oscillations in heart rate, resulting in higher variability and more gradual patterns in lowering of heart rate during eupnea.

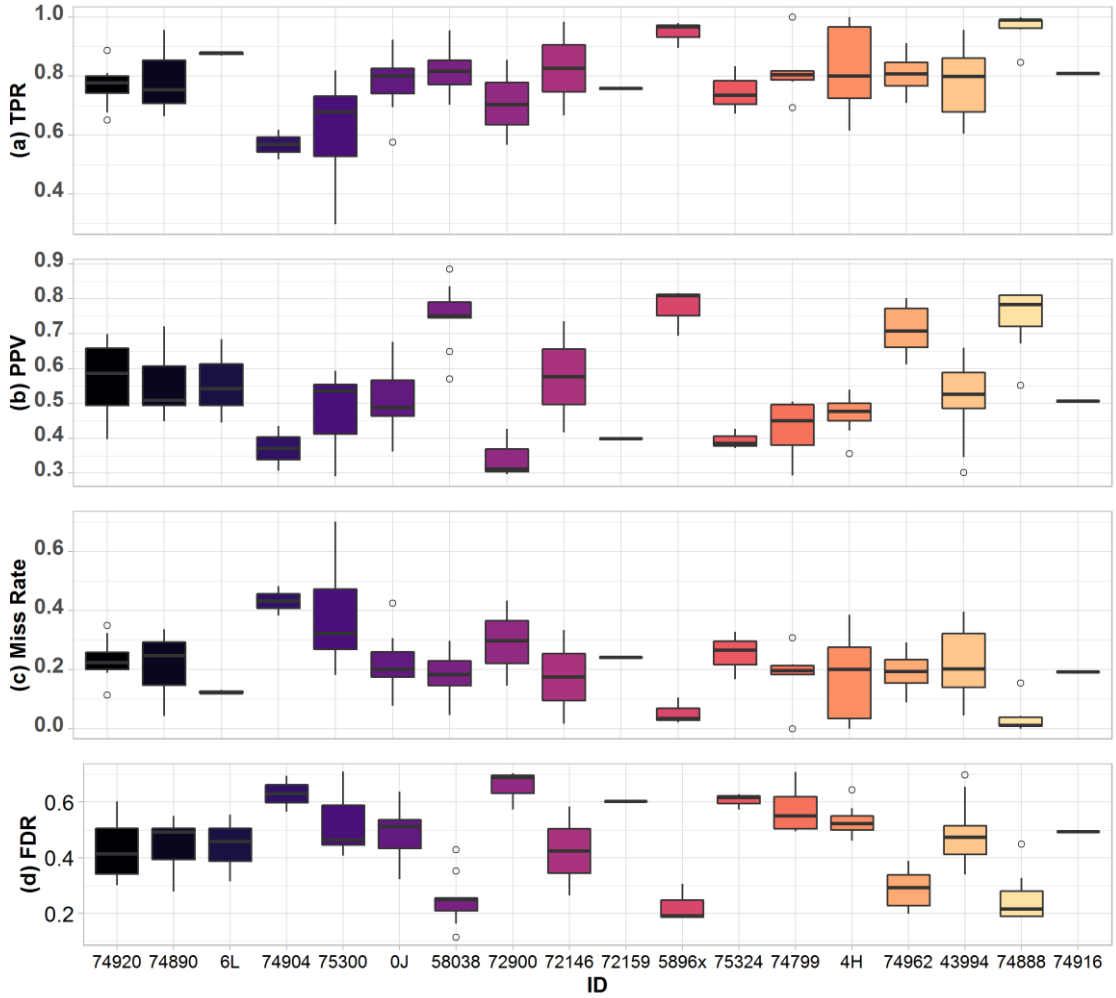


Figure 6.7: Boxplots illustrating variability in the ability of breath detection from animal-borne accelerometry across individuals (n_{ind} = 18) as compared to video-decoded breaths. Algorithm success is indicated by (a) true positive rates (TPR), (b) positive predictive value (PPV), (c) miss rate, and (d) false discovery rate (FDR) for each individual included in the video analysis.

6.3.3 – Functions of apnea

Periods of apnea were classified as being periods of inter-breath intervals longer than 11 seconds in duration. ACC-derived apnea periods matched well to those from video footage, corresponding to -3.59 ± 6.7% difference between the two measures as determined from Bland-Altman analysis (**Figure 6.8**). Females spent 24.8 ± 7.7% of time resting in a state of apnea measured across all segments of ACC data, though some females were more consistent than others (**Figure 6.8**). Females who spent more time in apnea, expressed as the mean proportion of time spent in apnea during 15-min segments per day, were found to have a significantly lower estimates of resting metabolic rate (estimated via min-f_H; β = -68.33 ± 21.72, p = 0.002;

Figure 6.9A) and significantly lower estimates of daily energy expenditure (estimated via mean-f_H; $\beta = -71.21 \pm 19.38$, $p < 0.001$; **Figure 6.9B**). No relationship was found between time spent in apnea and either of the fitness outcome proxies. However, a significant polynomial relationship was found between daily maximum temperature and mean daily proportion of time spent in apnea at rest ($\beta_0 = 0.25 \pm 0.006$, $\beta_x = 0.13 \pm 0.05$, $\beta_x^2 = -0.31 \pm 0.05$, $p < 0.001$; **Figure 6.9C**). Mean daily apnea was found to be highest between 7-12ºC, but rapidly decreasing above and below this range. Individuals who were able to spend the greatest amount of time in apnea (expressed as the maximum time spent in apnea during 15-min segments over lactation) were found to increase their haematocrit (%PCV) over lactation (**Figure 6.10**).

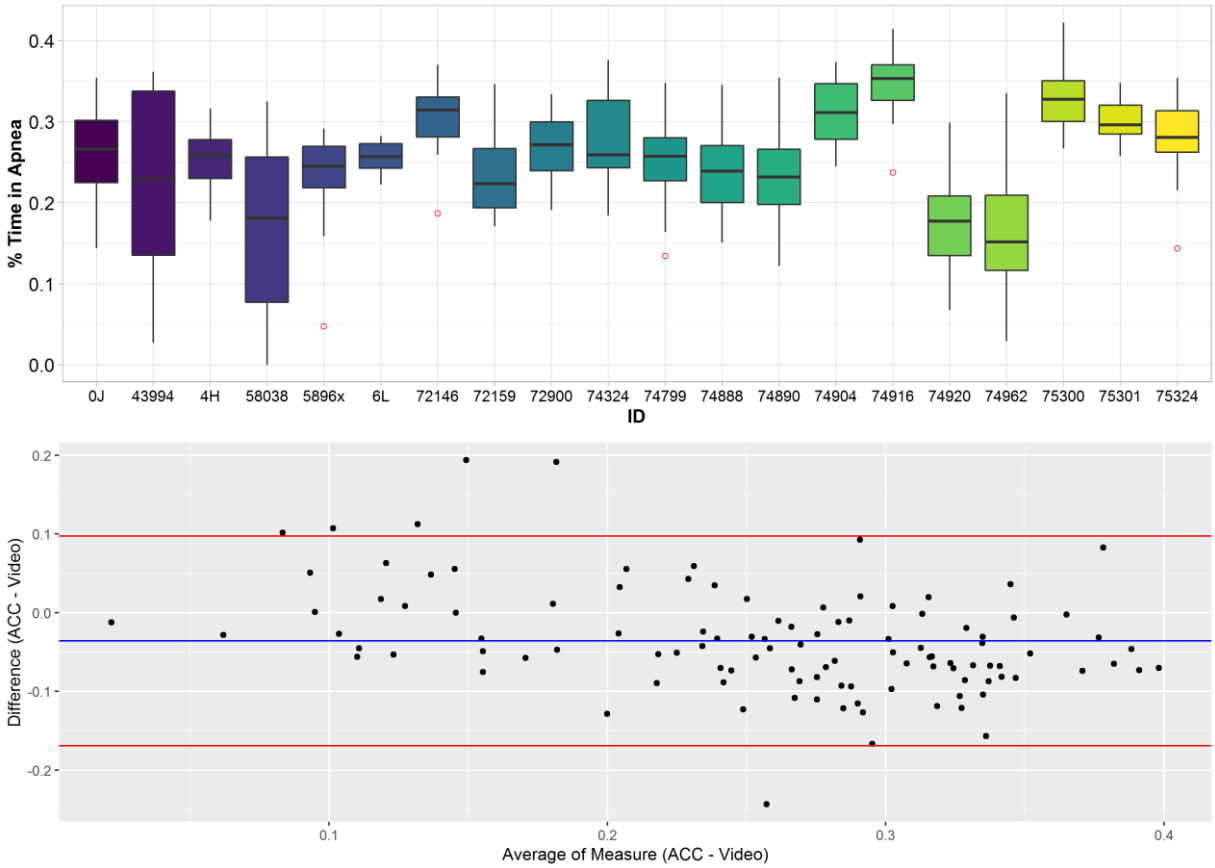


Figure 6.8: Box plot of the percent time spent in apnea as determined through accelerometer-derived breaths across all 15-min segments for each individual as well as the agreement between ACC-derived apnea and video-derived apnea using Bland-Altman methods (blue line indicates the mean difference between the two measures, while the red lines indicate 95% confidence).

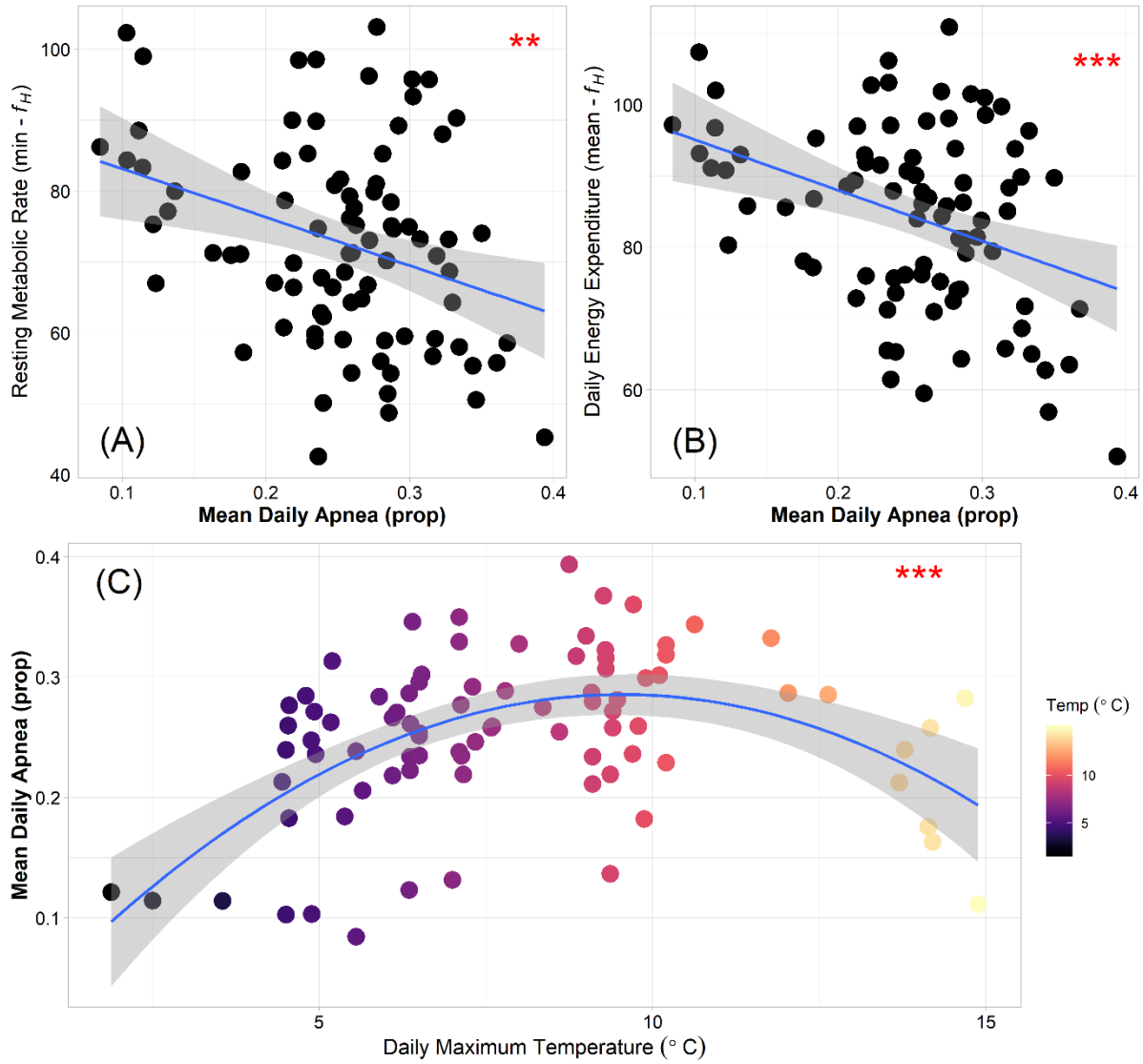


Figure 6.9: Relationship between time spent in apnea against energy allocation (A, B) and environment (C). Spending a greater average amount of time in apnea in a given day (mean daily apnea measured across all 15-min segments of ACC-derived apnea) translated to both a significantly lower estimated resting metabolic rate (A, min-f_H; $\beta = -68.33 \pm 21.72$, $p = 0.002$) and significantly lower overall daily energy expenditure (B, mean-f_H; $\beta = -71.21 \pm 19.38$, $p < 0.001$). Daily maximum temperature was best found to predict time spent in apnea (mean daily apnea) through a significant polynomial relationship (C; $\beta_0 = 0.25 \pm 0.006$, $\beta_x = 0.13 \pm 0.05$, $\beta_x^2 = -0.31 \pm 0.05$, $p < 0.001$). Significance is noted by stars on each plot.

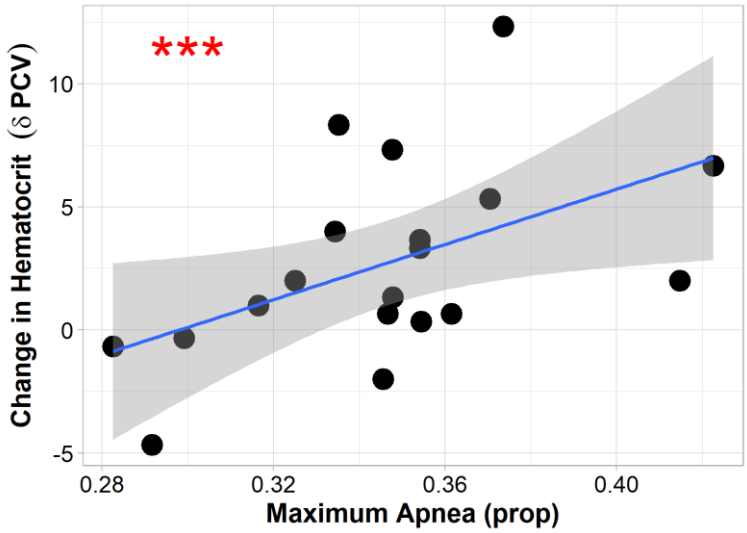


Figure 6.10: Relationship between the change in haematocrit between final and initial capture events as a response to the maximum time spent in apnea over lactation. Individuals who could spend the longest time in apnea (maximum value of time spent in apnea over lactation, as a proportion of a 15-min segment) were found to have significantly increased haematocrit (δ PCV) values over lactation ('***'; linear regression value indicated by blue line with grey confidence intervals).

6.4 – Discussion

In a novel use for externally-mounted accelerometers, individual breaths and periods of apnea were reliably detected and characterized during periods of rest in a wild population of grey seals. While the signal may have been contaminated with small peaks associated with minor adjustments in posture, using this method to detect differences between apnea and eupnea while at rest on land is extremely valuable, especially when individual heart rate is being recorded simultaneously. The same accelerometers were not successful in sensing heart rate, likely as a function of inadequate sampling rate and placement. The method presented here for detecting individual breaths and apnea is extremely flexible towards application in other species, marine or terrestrial, and that could characterize such physiological data without the need for visual observation. Using these methods, the potential drivers and consequences of apnea were also examined for the first time. Females who spent more time in apnea were found to have significantly lower resting metabolic rates and overall lower daily energy

expenditure, potentially benefiting from apnea as an energy saving mechanism. Time spent in apnea while resting exhibited no link to short-term fitness outcomes within a season, however, suggesting that energy saving is part of an overall trade-off in balancing energy usage. In addition, more time spent in apnea also translated to a significant increase in haematocrit over lactation. Time spent in apnea was also found to respond in a polynomial fashion to changes in temperatures, indicating that females may also be exploiting apnea as a water conservation method as well as an energy savings mechanism until maximum temperatures surpassed 12ºC.

6.4.1 – Breathing rates from accelerometers

Individual breaths were detected well across most females, though several individuals were poorly classified overall. The largest source of false positive breaths detected from the accelerometers were likely due to small movements as would be found in behaviours like Alert and Comfort Movement behaviours (see **Chapter 2.5.1-A.2 and A.3-CM**) that would have been missed by the criteria used to select the accelerometer segments. Attempts to remove these through a secondary filter did little to remove these false positives, resulting in only about 1% difference in FDR between filtered and unfiltered datasets (**Table 6.2**). More stringent filters or shorter accelerometer segment lengths may have improved individual breath detection overall. However, the use of segments of 15 minutes in length was chosen to capture a number of individual cycles between apnea and eupnea, while also biasing efforts towards capturing individuals who were truly at rest. Event detection as a whole has remained a very difficult aspect of biologging and success can be subject to the criteria used to describe the event (Brown et al. 2013). Usually, events are detected through visual analysis of tagging data channels and are most often used to detect events such as prey capture attempts either through peaks in acceleration (e.g. Skinner et al. 2010; Viviant et al. 2010; Owen et al. 2016), wiggles at the bottom of dives (e.g. Halsey et al. 2010), temperature fluctuations within the stomach (Ponganis 2007; Watanabe and Takahashi 2013; Sauvé et al. 2014), and confirmed with video

footage or acoustics (Davis et al. 2013; Watanabe and Takahashi 2013; Bom et al. 2014; Volpov et al. 2015a; Wijers et al. 2018). However, if this process is automated, slight alterations in the criteria used to define a 'real' event may cause large shifts in those data correctly classified. The method presented here is obviously limited to only periods of immobility while on land and would likely not function well in aquatic contexts, nor while an individual is active. However, it does present a useful case study toward the implementation of such automatic event detectors for terrestrial mammals. Previous work, while in detecting heart rate during periods of movement was unachievable, still found use in detecting heart rate and breathing rate following exercise to be a useful measure of metabolic capacity and recovery (Signer et al. 2010, 2011; Halsey et al. 2019).

Individual patterns in breathing rate can be an important indicator of metabolic and aerobic capacity as well as recovery from exercise (Fahlman et al. 2016; Garcia Párraga et al. 2018; Isojunno et al. 2018). The ability to detect individual breaths without the need for *in situ* observation represents an incredibly useful tool for a wide variety of applications for inferring physiology in terrestrial contexts. Many existing or previously deployed tags include accelerometers in a wide variety of taxa (Byrnes et al. 2011; Brown et al. 2013; Wilson et al. 2014; Maresh et al. 2015; Wang et al. 2015; Soltis et al. 2016; le Roux et al. 2017; Wijers et al. 2018). The methods presented here are easy to implement, adaptable to a variety of tag placements and taxa, and not computationally intensive to extract; users simply need to decide which data channel might contain the most information about chest expansions in order to reliably derive individual breaths, or a lack thereof. For example, forward displacement of dynamic acceleration was the most reliable in the current investigation, but in a collar-mounted accelerometer (McClune et al. 2014; Wang et al. 2015; Fehlmann et al. 2017), the norm of jerk or VeDBA may be a better indicator of breaths while an animal is at rest since the direction of displacement vectors likely change over time for non-static sensor mounts. In addition, the use

of a this peak detector algorithm presents a very simple method for extracting these peaks in chest expansion, but could easily be adapted or swapped for another algorithm or custom written code.

In the current study, comparing ACC-detected breaths to real-time recordings of heart rate presented a very useful tool for studying apnea and eupnea on land in relation to heart rate parameters (e.g. **Figure 6.6**). However, when validating these detected breaths, it was noted that several tags suffered from moderate to severe clock drift resulting in measurable offsets from the heart rate data of up to 100 s and was not consistently or even linearly decaying over time. While these could be manually adjusted against visually-decoded breaths, this difference could be critical if accelerometer-detected breaths need to be matched to other data channels on separate loggers or transmitters and prohibited the current investigation from relating heart rate to breaths across all accelerometer segments. Several methods exist for correcting clock drift associated with GPS and could be applied to tags that can be communicated with remotely (Banerjee et al. 2012; Volpov et al. 2015b; le Roux et al. 2017).

6.4.2 – Functions of apnea on land

Phocid seals and other diving mammals have evolved a variety of traits that have allowed them to exploit resources in the marine system. In order to dive to extreme depths, marine mammals have extended the mammalian dive reflex to allow them to hold their breath for lengthy periods of time in order to exploit variable prey patches throughout the water column as deep as several thousand meters, without reaching their aerobic dive limit in many cases (Horning 2012; Williams et al. 2015; Andrews and Enstipp 2016; Kaczmarek et al. 2018; Shero et al. 2018). The ability to stay aerobic during these extreme dives is aided by efficient oxygen uptake and storage throughout the body, marked bradycardia during breath hold, as well as other adaptations such as streamlined body form, ischemia to certain regions of the

body, swimming economy, and a collapsible lung space to decrease buoyancy (Schmidt-Nielsen 1972, 1997; Williams et al. 2015). These physiological traits develop quickly after birth and through the first few years of life in order for individuals to expand the vertical niche of prey acquisition (Horning and Trillmich 1997; Burns 1999; Noren et al. 2005; Richmond et al. 2005; Carter et al. 2017) and do not appear to decline with age (Hindle et al. 2011). Haematocrit is a coarse measure of oxygen storage capacity and was measured here as %PCV in whole blood. The increase in haematocrit over lactation was found to increase in individuals who were able to spend the greatest proportion of time in apnea during rest over lactation (maximum apnea). Castellini et al. (1986) found that haematocrit increased following periods of extended apnea. The results presented here also points to a link between aerobic capacity as expressed on land to diving ability at sea. This reflects established links between endogenous calculated aerobic dive limit parameters (such as haematocrit) and diving ability in a variety of other pinniped species (Horning and Trillmich 1997; Noren et al. 2005; Richmond et al. 2005; Hindle et al. 2011), but should be explored further. Building links between aerobic capacity while on land may serve as a robust indicator of diving ability, without the need to capture animals at sea. Haematocrit, however, also varies with the hydration state of the individual (Schmidt-Nielsen 1997). If this apparent signal relating apnea to changes in haematocrit across lactation is in fact related to hydration state, an increase in haematocrit over lactation may actually reflect an individual who is approaching a relatively dehydrated state while on land. If this is the case, spending more time in apnea in this case does appear to provide significant water conservation benefits.

While extended apnea at sea certainly benefit diving ability, it is unclear why it persists on land and has only been noted in a few studies previously (Castellini et al. 1986, 1994; Castellini 1996; Andrews et al. 1997). When examining the mean time spent in apnea over resting periods, significant relationships were found between estimated proxies for resting

metabolic rate (min-f_H) and total daily energy expenditure (mean-f_H). Study females who spent significantly more of their resting periods in apnea for each day (mean daily apnea) were found to have a significantly lower resting metabolism, or background process costs, and a significantly lower daily energy expenditure (e.g. Portugal et al. 2016; Halsey et al. 2019). This suggests that these female grey seals may be benefiting from the ability to enter apnea as a mechanism for energy savings. While it was not possible to directly compare the proportion of bradycardia associated with these apneic periods due to clock drift, females who are capable of lowering their overall energy consumption by decreasing both heart rate and respiration may be better able to balance the energy trade-offs associated with their contracted and high-demand lactation period. In addition, daily maximum temperature across the colony was found to be associated with mean daily apnea rates. The proportion of apnea steadily increased as temperatures increased towards about 12^{o}C and then appear to drop steeply above that temperature. There are several potential reasons for this. As noted in **Chapter 5**, individuals experience a steady increase in the cost of background processes and daily energy expenditure as temperatures increase. Females may be using apnea as a mechanism for ameliorating increasing thermoregulatory burdens. The extreme metabolic output of lactation in these grey seals (e.g. Mellish et al. 2000) may have shifted their internal thermoneutral zone such that ambient temperatures approaching 15^{o}C (maximum ambient temperature measured in the current study) may be reaching their upper critical temperature. While we may not be able to directly measure this, increasing apnea as temperatures rise may also be a mechanism for water conservation. As previously discussed, grey seals have only two options to replenish water stores while not feeding: travel to ephemeral pool sources to drink fresh water or rely on fat catabolism to free up internal body water (Twiss et al. 2002; Stewart et al. 2014). As a result, respiring less or saving energy while at rest may reduce the need to travel to a pool or free up body water over time, but only to a certain threshold. Individuals appear to be able to use

apnea up until 12^{o}C to likely both conserve water and energy stores, but may no longer be able to benefit from apnea above this temperature and instead rely on alternative routes, such as commuting or drinking, for thermoregulation and water balance. Energy and water conservation as drivers of apnea may be good indicators of how well individuals can balance energy trade-offs over time and should not be considered as two mutually exclusive benefits of apnea.

6.4.3 – Limitations of externally mounted heart-rate detection

Several studies have found success in determining heart rate using accelerometers. While many of these were developed for use in clinical situations (Castiglioni et al. 2007; González-Landaeta et al. 2007; Phan et al. 2008; Pandia et al. 2010; Bicen and Inan 2018), several have found success in determining heart rate for wild animals using both internal and external configurations (Pearson et al. 1998; Signer et al. 2010). Internally placed accelerometers within the rumen of several species of ungulates have reliably extracted heart rate up to several months following deployment and present an incredibly useful method for tracking physiology in wild animals (Signer et al. 2010, 2011; Turbill et al. 2011). What these successes have in common is that they are able to sample acceleration at very high rates, often sampling in excess of 100 to 1000 Hz. Theoretically, a sampling rate of 50 Hz should have been able to capture the frequency of heart rate, given that the Nyquist frequency (25 Hz) of the current tagging configuration should exceed the highest heart rate measured for these grey seals (200 beats min^{-1} ~ 3 Hz; Lynn 1982; Cadzow and Van Landingham 1985; Taylor 1994). However, sampling at this lower resolution meant that aliasing may have contaminated the signal with excessive noise and could not be properly removed through filtering (Shannon 1998). Aliasing, simply put, means that any frequency elements of a signal above the Nyquist frequency will cause distortions when the signal is resampled, and in this example, contaminate the clear peaks with noise as can be noted in **Figure 6.1** (Cadzow and Van Landingham 1985).

Sampling at a higher rate would allow for the correct extraction of heart rate through low-pass filtering, also known as an anti-aliasing filter, followed by subsequent amplification through a band-pass filtering of the signal (Shannon 1998). While the same protocol was attempted in the current study, the frequency resolution was not fine enough to properly remove aliasing from the peak detector algorithm used here.

Several practical constraints may have also limited the interpretation of the signal. Ideally, vibrations from the heart are best measured from the chest wall, whereby the force of the ejection of blood from the heart would be maximally sensed with little delay in the signal. The placement of the accelerometers in this study, however, would have sampled the force of movement from the descending aorta (Rommel and Lowenstine 2002). The force associated with this signal (cardiac output) should scale accordingly with the product of heart rate (frequency) and stroke volume (ml; Schmidt-Nielsen 1997). As a result, only higher heart rates were detected and could be the result of changes in stroke volume and subsequent scaling of the kinetic energy of the fluid through the aorta (Schmidt-Nielsen 1997). This was likely compounded by the influence that blubber may have in amplifying or softening the signal to the accelerometers. The detector algorithm was more successful in detecting heart rate early in the season, when seals have markedly thick blubber layers, compared to later in the season (**Table 6.1**). Therefore, thicker blubber may act to amplify heart sounds rather than insulating it, though the confidence intervals around estimates early in the season were still very large. Other configurations of exteriorly mounted animal-borne accelerometers may see better results than obtained here, such as on the chest wall. Even though heart rate was not reliably extracted from accelerometers, heart rate as determined from externally mounted heart rate monitors are still a viable method for tracking heart rate, as is clear in the current study.

6.4.4 – Conclusions

Overall, individual breaths were reliably extracted from accelerometers mounted on the back across a variety of individual female grey seals and contexts. The method presented here represents a useful starting point for many other terrestrial taxa in obtaining breathing rates without the need for *in situ* observations. Within grey seals, this allowed for a fine-scale analysis of how maintaining periods of regular apnea on land may be co-opted for energy savings and water conservation, while responding to environmental and energy management drivers over the high-demand period of lactation. While heart rate was not extracted well from accelerometers mounted on the back, future deployments may find success using accelerometers mounted closer to the heart and chest wall.

Chapter 7:

General Discussion

7.1 – Summary of main findings

Grey seals, like many other pinnipeds, live a duality by being adapted to both aquatic and terrestrial environments and as a result must handle a wide variety of constraints on ecology, behaviour, and energy expenditures that are beneficial for living the water but are not necessarily conducive to spending time on land. As a result of these constraints, the capital breeding system used by grey seal females offers a reduction of the time required to be hauled out on land, but at the cost of narrow margin of error for successfully raising a healthy pup while not damaging themselves in the process. Changing climates in the temperate regions that grey seals inhabit may further perturb this fine balancing act. The main goal of this thesis was to use new technologies, specifically animal-borne accelerometers and heart rate monitors, to examine in detail how individual grey seal mothers remain within these narrow margins. By examining how differences in behaviour, energy usage and conservation, and fitness are not only balanced in a wild population, but also how the effects of stress-coping styles and environmental pressures can modulate these responses, can usable baselines be established to measure future change.

This thesis has used a wide variety of recently developed technologies applied in a novel fashion to elucidate fine-scale time and energy trade-offs in lactating grey seals, overcoming many of the limitations of traditional behavioural observation studies. Through the lens of animal-borne accelerometers and heart rate monitors, this study has shown that individuals carefully manage their time and energy over lactation in such a way that allows them to maintain the high energy throughput of milk production and investment into offspring without damaging themselves in the process. New algorithms developed here for accelerometers can aid the remote monitoring of not only behaviour during lactation in this species, but also in detecting breathing and apnea in a wild context which could be applicable to a wide variety of aquatic and terrestrial species. This study highlights how differing

physiological and behavioural strategies are still able to balance the demands of lactation in the face of a changing environment. However, this thesis has also revealed that personality differences, in the form of stress-coping styles identified from inherent differences in resting heart rate variability, have an extensive influence on not only the way an individual spends their time, but also with respect to their overall energy budget, energy management, and fitness outcomes over time.

7.1.1 – Main findings

1) Accelerometers can identify and classify the bulk of a female grey seal's activity budget *in situ* (~ 90% to time-activity), achieving a coarse-level resolution of mostly static behaviours using the robust random forest algorithm.

2) Accelerometers placed on the torso of an individual show the greatest promise for extracting behaviour in female grey seals, but potentially at the cost of a loss of information for behaviours relying specifically on the movement of the head, such as vigilance-type behaviours.

3) Accelerometers can potentially be used to measure individual preferences in lateralization, as noted by the novel evidence presented with respect to a form of mother-pup interaction, Flippering pup.

4) Female grey seals exhibit similar trade-offs to other lactating mothers, where individuals are keenly focused on balancing time spent Resting (conserving resources) against remaining vigilant (Alert). These behaviours were traded-off throughout the pup's developmental period, differing between pup sexes, and varying with approximate colony density.

5) Resting heart rate variability, as a measure for stress-coping style, appears to play a significant role in a female's activity budget over lactation. Proactive females typically spent more time Alert and less time Resting, on average, than females who exhibit reactive tendencies.

6) Presenting/Nursing effort appeared to be largely fixed within an individual over lactation, indicating that consistency in this behaviour may be highly selected for in capital breeders and only varied with respect to a female's location on the colony and time, roughly equating with differences in density.

7) Grey seal females maintain a compensation energy management strategy during lactation, balancing metabolic and background process demands against energy

required for behaviour, as determined through metrics of heart rate. Individual strategies of energy usage and management appear to remain consistent between years.

8) Female grey seals also apparently trade-off time inactive and engaging in low-level activity with respect to energy usage. Spending more time inactive resulted in a lower metabolic demand, but at a greater energetic cost for activity. The opposite was found for spending more time engaged in low-level activity. This suggests that activity level state-switching after an extended period of inactivity, and depressed metabolic rate, accrues a higher cost than returning to inactivity from an active state.

9) Using accelerometers as the sole measure of energy usage in a largely inactive model species is inappropriate and does not capture any of the trends or variability associated with daily energy expenditure during this phase of pinniped life history, following the removal of time from the relationship.

10) Grey seal females appear to be able to handle increasing demands of thermoregulation by apparently lowering their 'background' processes and overall daily energy expenditure. While ambient temperature appeared important for predicting activity, no significant relationship could be found.

11) Stress-coping styles also play a large role in the overall scope and plasticity of energy management strategies. Proactive females tended to have higher metabolic costs and daily energy expenditure than reactive females. Proactive females also had higher mass-transfer efficiencies and mass loss rates than reactive females over lactation. Reactive females, however, were much more variable in these fitness outcomes.

12) Accelerometers, given their external scapular placement and sampling configuration in this study, were unable to accurately and consistently detect heart rate from arterial vibrations on the back.

13) Individual breaths were readily recognized using a newly applied event detection algorithm and accurately detected when compared to video footage. Event detection, however, varied greatly by individuals, overestimating breaths in individuals who regularly engaged in low energy behaviours.

14) Remote breath detection allowed for accurate measures of periods of apnea while individuals were at rest. This has the potential to extend studies of apneic and eupneic breathing in a wild animal without the need for video footage, assuming clock drift can be properly accounted for across multiple sensor channels.

15) Apnea on land appears to confer significant energy saving benefits during lactation by allowing individuals to reduce their metabolic overheads and daily energy expenditure.

16) Apnea on land also may allow individuals to conserve water and address thermoregulatory demands, by increasing time spent in apnea as temperature increases towards 11°C. This same relationship also appears to indicate that time spent in apnea steeply declines after this point, suggesting that females seek other avenues for thermoregulation and water needs at higher temperatures.

17) The ability to positively increase haematocrit over lactation was also significantly associated with a maximum capacity to spend time in apnea. This suggests that differences in diving ability related to blood and muscle oxygen stores or hydration state may drive or be driven by the ability to extend apnea, but needs further validation.

The remainder of this chapter will discuss the broader implications of this study as well as the links between results across chapters and how they may relate to larger topics of ecological physiology, personality differences, evolutionary constraints, and the use of these accelerometry and heart rate techniques in other species. The limitations of the current study will also be discussed in detail. Discussions of how this work could be extended to other questions relating to behaviour outside of the lactation period in grey seals, physiological measurements and their application to other species and systems, ecological and evolutionary constraints for stress-coping styles, and broad considerations with the increasing pressures of a changing climate and pace-of-life are also included.

7.2 – Technical and contextual considerations for interpreting behaviour

7.2.1 – Combining multiple data channels for behaviour

Accelerometers have provided an incredibly powerful tool for tracking movement over time, here in **Chapter 3** and elsewhere in a variety of studies and contexts (Brown et al. 2013). Tracking behaviour in shy or difficult to access individuals and populations has revealed some surprising aspects life history and mechanisms of behaviour that were previously unknown. Accelerometers mounted for short periods on large Mysticete whales have revealed the fine-

scale kinematics of lunge-feeding behaviours (Goldbogen et al. 2006, 2013; Owen et al. 2016), while those attached to captive elephants revealed different postures associated with apparent affective states (Wilson et al. 2014). Using accelerometers here, novel evidence of lateralization was clearly present with respect to mother-pup interactions (**Chapter 3**), while full activity budgets indicated that female grey seals do not trade-off time spent in effort towards Presenting/Nursing over the course of lactation and instead balance time Resting against time Alert (**Chapter 4**). While **Chapter 3**, **4**, and **5** have already discussed limitations of accelerometers as a sole measure of activity, it is important to consider capturing behaviour from multiple data channels in order to accurately and consistently classify more complex behaviours, such as locomotion or social interactions. When the behaviours detected from these tags are properly calibrated to energy usage (e.g. $\dot{V}O_2$), accelerometer data can provide useful measures of the average energy expended per behaviour(Williams et al. 2004; Wilson et al. 2006; Halsey et al. 2009b, 2011a; Qasem et al. 2012; Lyons et al. 2013). Even without direct calibration, the use of accelerometers in conjunction with heart rate revealed qualitative, yet informative, relationships between energy partitioning and activity levels in **Chapter 5** for the grey seal mothers used in this study. Accelerometers have also provided an incredibly powerful companion to other tagging data, such as gyroscopes or magnetometers, and metabolic calibrations to further elucidate the relationships to energy usage in other studies (Wilson et al. 2006; Fahlman et al. 2016). Further examples of this include seasonal energy partitioning in sea snakes and marine turtles assessed though temperature-specific metabolism and movement data (Fossette et al. 2012; Udyawer et al. 2017), escape responses in a naïve cetacean species using diving, movement and heart rate data (Williams et al. 2017), and the energetic strategies associated with the highest migration in the world by combining long-term measures of heart rate data and three-dimensional movement data (Bishop et al. 2015c). Future deployments of accelerometry should consider applying tags sampling multiple channels of

data to further enhance the resolution of behavioural and physiological relationships of pinnipeds and other species.

7.2.2 – Sociality and dyads

While accelerometers have provided unprecedented access to behaviour, several areas of interest for behavioural research could be developed further. More specifically, accelerometers largely lack the ability to measure or track behaviours associated with social context. Mechanistically, these types of social behaviours, such as those measured in grooming behaviour in baboons, are difficult to separate between the actor and recipient (Fehlmann et al. 2017), and are often random in their mechanistic execution. In **Chapter 3**, social and aggressive behaviours were almost entirely missed in all sampling and machine learning approaches. The only interaction behaviour that was classified well was that pertaining to the mother-pup interaction, Flippering pup (**2.5.1-A.4-FP**), as it was both consistent in mechanistic space and remained as a measurable state over extended periods of time. In addition, no research has attempted to use accelerometers in the context of dyads, which has limited the interpretation of social and interactive behaviours to a single actor. Remote observation of behaviour in social groups may be even more problematic. Deploying multiple tags on stable social groups may be useful in remotely identifying behavioural interactions among conspecifics, however few species exhibit such stability and group deployments have only really been attempted with more generalized movement data (e.g. Crofoot et al. 2008, 2010). Researchers must also balance the costs of deploying multiple tags on a single family or social group against the desire to sample the larger population. While evidence of lateralization was detected in Flipper pup behaviour in this thesis, no studies to date have attempted to investigate these aspects of lateralization of social and aggressive behaviour through the lens of accelerometry, despite its usefulness in determining repeatable body positions. Lateralization also likely exists in aggressive or non-pup social contexts in

grey seals and other mammals, but has not been evaluated and may remain difficult to evaluate without dyadic tagging efforts as lateralization specifically relies on the relative positioning and interaction between two individuals (e.g. Hill et al. 2017). Conflict and sexual behaviour are popular aspects of behavioural study (Twiss et al. 1998, 2007; Bishop et al. 2014, 2017; Debecker et al. 2016). Without a reliable or affordable method for deploying and tagging individuals within these social contexts as well as the possibility of damage to larger units during conflict or mating, studying animal conflict and sexual behaviour may not be possible in the current state-of-the-art of accelerometry.

7.2.3 – Technical and scale considerations for interpreting behaviour

Questions using accelerometers must balance the ability to interpret behaviour across a variety of spatial, physiological, and temporal scales, including both biomechanical and time-specific considerations. Accelerometers are specifically limited by only sensing and distinguishing behaviour when biomechanics allow sufficiently different signatures in accelerometry space. Locomotion, as an example, is consistent in its interpretation in quadrupeds as motion generated from limbs is allometrically scaled across body sizes (Heglund and Taylor 1988; Hoyt et al. 2006; Shepard et al. 2008; Gleiss et al. 2011). The overall frequency of movement too, often expressed as stroke or stride frequency in marine and terrestrial animals, respectively, is also found to scale with body size, but does not necessarily dictate speed of movement (Sato et al. 2007). This has made locomotory behaviours an incredibly popular behavioural state to extract from accelerometers (Sato et al. 2003; Williams et al. 2004, 2017; Karantonis et al. 2006; Ponganis 2007; Robert et al. 2009; Nielsen et al. 2010; Soltis et al. 2012; Kölzsch et al. 2016). Locomotion in pinnipeds on land has also been found to be very stereotypical over prolonged commuting periods (Tennett et al. 2018), but was not consistently classified in **Chapter 3** due to its brevity in time and was unable to be separated by context. As a result, if two behaviours of different contexts are too similar in

mechanics, they are essentially inseparable in the eyes of an accelerometer which limit the ability to interpret these behaviours. For example, one cannot separate Nursing behaviour from Presenting in any of the measures of behaviour used in this study, both in the context of decoding video footage (**2.5.2**) nor with respect to the activity derived from accelerometers. As a result, this behaviour can only be considered total effort rather than a distinct set of behaviours relating to milk exchange with the pup (**Chapter 4**). In addition, behaviour often involves instantaneous events, such as head movements relating to pup checks (**2.5.1-A.2-PC**) or biting and lunging (**2.5.1-B.2-BI**). As noted in **Chapter 6**, event detection is one of the least developed aspects to machine learning and accelerometer-derived behaviour (DeRuiter et al. 2013; Shorter et al. 2017). Since many of these instantaneous behaviours mechanistically appear the same in accelerometer feature space, they are not detected as individually unique behaviours. Applications of accelerometry, as a result, must consider that behavioural data generated across high temporal resolution will almost certainly be at a loss of nuanced contextual information.

Time also plays a crucial role in extracting behaviour from accelerometers. In the current study, all behaviours were extracted on a second-by-second scale in order to attempt to capture behaviours that last less than one second. However, if sampled over several seconds to track behaviour as states lasting for longer periods, more accurate classifications of states may have been achieved, at the loss of any detailed behavioural information. Collecting behavioural data over long stretches of time will require a trade-off in time and frequency resolution with deployment times. Deploying tags at a lower sampling rate means that behaviour can be collected over longer periods and possibly with better resolution for locomotory behaviours (e.g. less noise), but instantaneous and minor movements will be missed entirely (**Chapter 3**). Conversely, detecting heart rate through externally-mounted accelerometers (**Chapter 6**) at a higher sampling resolution would have also prevented aliasing

and noise contamination, but at the cost of a shorter deployment on study individuals. Researchers must consider the desired outcome for the acceleration data in order to balance deployment duration, resolution of desired behaviours, and effects of resampling for future efforts.

While troublesome to diagnose, clock drift presents a difficult problem to address when attempting to link up several channels of data over time, such as matching behaviour from accelerometers to video or heart rate data. As previously discussed, this prevented the ability to match up breathing rate and apnea data in **Chapter 6** to real-time heart rate data without manual adjustment, though whether this was caused by the accelerometer tags or heart rate software remains to be seen. Clock drift is simply when a clock of interest does not run at the same rate as real time. In mechanical clocks, this means that small imperfections in the movement mechanisms will cause a random drift away from the actual time. Digital clocks as would be housed in the accelerometers and other tags use very low clock frequencies associated with a microcontroller in order to time the execution of data collection (Karantonis et al. 2006; Signer et al. 2010). When this microcontroller slows or malfunctions, clock drift will occur in a tag. However, this delay would not necessarily be readily apparent in the way that timestamps are assigned *post hoc* in the accelerometry software (AxyManager 2; see **Chapter 2**). It is likely that 'missing' data were simply ignored relative to their time stamp and skipped. In the case of GPS telemetry devices, this can often be corrected by running internal controls or by communicating with the device periodically in order to synchronize clocks (e.g. le Roux et al. 2017). In addition to drift, significant aliasing was noted in **Chapter 6** when attempting to extract heart rate data from accelerometers. This can be corrected in future efforts by sampling at a much higher rate in order to properly filter out these aliased signals as previously discussed. While these recommendations and technical considerations are exceedingly obvious to specialized persons trained in engineering, many of these

considerations are likely ignored in recent applications of accelerometers by behavioural and physiological ecologists; only a handful of referenced studies mention these considerations (Le Boeuf et al. 1996; Banerjee et al. 2012; Volpov et al. 2015a; Fujioka et al. 2016).

7.3 – Energy management strategies

7.3.1 – Variability and fluctuation of energy management strategies

In investigating energy trade-offs in female grey seals, significant and repeatable relationships with respect to energy management strategies were noted (**Chapter 5**). Female grey seals, as a population and as individuals, were found to exhibit patterns of energy usage that agree with what is known as a compensation model of energy management (Careau 2017); females prioritize consistent daily energy expenditure by trading-off energy partitions allocated to background metabolic processes against energy partitioned towards behaviour. Several of these underlying aspects of energy partitioning within compensation energy management have long been implicitly evaluated with studies of lactation energetics and behaviour in pinnipeds (Thompson and Nicoll 1986; Kenagy et al. 1989; Trillmich 1990; McLean and Speakman 1999; Mellish et al. 2000; Therrien et al. 2008) and the foundational concepts of income and capital breeding across species (Schulz and Bowen 2004, 2005; Stephens et al. 2009). These previous studies extracted energy usage patterns on coarse timescales, noting relationships between start and end of a lactation period or sampling every few days, often shifting the focus towards examining the end products of energy usage and allocation such as milk energy density (e.g. Mellish et al. 1999a). This thesis is the first of its kind to use heart rate monitors and accelerometers in marine mammals to track these energy partitions at a fine temporal resolution across the duration of lactation, measuring energy management across each day without the need for extra handling events. As mentioned in Careau et al. (2008), measuring energy management allows for researchers to consider the importance of individual variability

in metabolism and other processes, previously considered to be ‘noise’ around species- and taxon-specific estimates energy usage strategies.

Investigating energy management on a daily basis can give a more robust picture of the intrinsic and extrinsic influences on energy partitioning, rather than simply measuring single point fitness outcomes or estimating net mass loss rate across lactation. More importantly, identifying energy management strategies also present the possibility to build population-level estimates of energy usage to use in comparisons across colonies, interspecies comparisons across phocids, and develop a more complete picture of the evolutionary pressures on managing energy usage across different stages of life history (Halsey et al. 2019; White et al. 2019). Several previous studies have commented on pinniped lactation strategies in terms of duration, milk energy outputs, and seasonal or geographical timing (Boness and Bowen 1996; Oftedal et al. 1996; Schulz and Bowen 2004, 2005). Namely, these studies indicate that a shorter duration of lactation results in a higher requirement for milk energy output and a more restrictive biogeographical range; species such as hooded seals (*Cystophora cristata*) who lactate for a mere four days have the highest milk fat content of any phocid and are restricted to breeding on high latitude ice floes (Mellish et al. 1999b). While these studies have focused on species-specific trends of lactation, the heart rate method used here to characterize energy management gives a more holistic picture of how energy partitions, specifically those broad partitions relating to background metabolism and activity, can be affected by external pressures across a wide variety of spatial and temporal scales within and between individual seals. Tracking heart rate parameters can reveal how energy demands fluctuate day-to-day and throughout an individual’s life history cycle. More importantly, however, this method allows researchers to evaluate whether or not plasticity in these energy partitions within individuals is balanced relative to the species-specific condition of energy management and daily energy expenditure during lactation (e.g. Halsey et al. 2019; White et al. 2019). For example, despite

likely being well within their thermoneutral zones, females grey seals in this study apparently lower background energy as temperatures rose (**Chapter 5**). The overall effect of this means that females may be able to selectively manipulate energy partitioning to absorb costs of other activities and environmental pressures in order to maintain the overall energy usage noted in other studies (e.g. Mellish et al. 2000).

Similar mechanisms of lowering certain energy partitions have also been noted in ungulate species in response to differing environmental pressures over a yearly cycle (Signer et al. 2011; Turbill et al. 2011), as well as how different waterfowl species trade-off the associated costs of moulting duration by means of sequential or catastrophic feather replacement in light of behavioural and energetic consequences over a year (Guillemette et al. 2007; Portugal et al. 2018). As a result, it is important to note that individual- and population-level energy management strategies are not necessarily invariable across yearly cycles or specific life history events (Halsey et al. 2019). Compensation likely only applies during fasting periods in order to efficiently utilize limited internal resources, such as during capital breeding or a period of catastrophic moult. Outside the breeding season, adult female grey seals, and other age classes such as juveniles or sub-adults, likely utilize an increased-intake, independent, or even performance strategy where daily energy expenditure may increase during foraging trips in order to meet the energetic requirements of a near-shore or pelagic lifestyle, depending on the time-scale considered (Beck et al. 2007; Carter et al. 2016, 2017; Halsey et al. 2019). Applying these methods used in this thesis during other stages of life-history or the yearly cycle may help to elucidate how grey seals and other pinnipeds manage and adjust energy expenditure across a variety of contexts. Energy management must remain plastic to the demands of the individual over time, else risk the deleterious effects of energy mismanagement.

7.3.2 – Energy management and water balance

Water has been a recurring theme in grey seal research over the breeding season (Pomeroy et al. 2001; Redman et al. 2001; Twiss et al. 2002; Stewart et al. 2014). As previously discussed, grey seals have several ways of meeting water demands throughout the year including water from prey consumption when feeding, metabolic body water, and drinking fresh water while on land (Stewart et al. 2014). Some females appear to be better able to maintain water balance during lactation than others, as noted by anecdotal observations of female movement and drinking habits throughout three seasons of study; several repeat capture females in dry areas of the colony did not appear to commute to pools, while adjacent females did regularly commute to freshwater sources. Across these three years, females experienced a wide range of temperatures (e.g. **Figure 5.10**) as well as other weather variables such as rain, resulting in several stable pools across the island almost completely drying up for a period during hot and dry weather (see **Figure 4.1**). While drinking was not well classified under the general 'Other' behaviour in **Chapter 3**, it would have given an interesting take on quantifying the need for external sources of water over lactation. The skull morphology of all pinnipeds has already evolved towards water conservation mechanisms through complex convolutions of turbinate bones and counter-current *retia mirabilia* in the nasal cavity (Rommel and Lowenstine 2002). Complex excretory systems elsewhere in the body, such as complex reniculate kidneys, also help to retain water in the marine environment (Schmidt-Nielsen 1997). While on land, however, it is unclear if these same adaptations reach a limit to their efficiency of retaining and maintaining water balance, especially when no longer feeding and relying primarily on body stores or ephemeral sources of fresh water.

This thesis uncovered several lines of evidence that may help understand water balance in lactating grey seals. Individuals appear to lower background and overall energy expenditure as temperatures warm, where the increasing costs of thermoregulatory demands likely also

drove a demand for water (**Chapter 5**). In addition, significant relationships were also noted with respect to spending more time in apnea as ambient temperatures increased which not only conserves energy stores, but also would likely confer some kind of water conservation mechanism (**Chapter 6**). Lactation is a route for water loss, regardless of species, and it is unclear if contracted lactation and consequently more intensive milk production is a mechanism for water conservation, as has been speculated in the past (Schmidt-Nielsen 1997). Desert-adapted species produce highly concentrated milk products, similar to phocid seals, and appear able to carefully manage water balance (Maltz and Shkolnik 1980; Nagy and Gruchacz 1994; Cain, III et al. 2006; Moses et al. 2012). While efficiency of hormone regulation likely dictates the efficiency and retention of body water, negative water balance is known to result in poorer quality milk in lactating species in the desert (e.g. Johnson 1965; Maltz and Shkolnik 1980; El-Tarabany et al. 2017), and may be the reason for the apparent decreased offspring size and condition at weaning in excessively dry years observed in this study where females terminated lactation earlier than expected (e.g. abandonments in 2016 and 2017, **Table 2.1**). A useful expansion to the work completed in this thesis would be to investigate how milk production in grey seals is affected by these environmental pressures. Since the measure of background energy expenditure (**Chapter 5**, min-f_H) likely also includes an energetic signal from milk production, it is unclear if minimizing background energy in relation to temperature is specific to metabolism, milk production, or both and should be investigated further. Concurrently, measuring hormones related to water turnover, such as antidiuretic hormone or aldosterone, may also shed light on how females balance water usage for milk production against her own water needs (Cain, III et al. 2006). How females balance differential inputs within these background processes in the face of climatic uncertainty will likely have the greatest impact on an individual's water balance and may explain the observed variation in drinking and heat tolerance.

7.3.3 – Lactation and thermoneutral zones

Previous work on breeding grey seals documented behavioural evidence of overheating on North Rona (Twiss et al. 2002; Stewart et al. 2014). Thermoneutral zones are defined as the range of ambient temperature to which an organism is either adapted or acclimated to where metabolism can be maintained at a constant rate. Bounding this thermoneutral zone, upper and lower critical temperatures (T_{uc} and T_{lc}, respectively) denote the limits where metabolic rates must increase in order compensate for increasing thermoregulatory burdens (Schmidt-Nielsen 1997). Differences in the insulative properties of thickened pelage or blubber can shift this range so that the thermoneutral zone for cold-adapted organisms allows them to tolerate much lower ambient temperatures than other organism of a similar body size without increasing metabolism (lower T_{lc}). Experimentally, thermoneutral zones are quantified by taking measurements of metabolism during controlled manipulations of ambient temperatures to determine these critical temperatures, often subjecting the individual to being constrained within a metabolic chamber for extended periods of time (Hart et al. 1959; Boily 1995; Liwanag et al. 2009; Kobbe et al. 2014; Rey et al. 2015; Udyawer et al. 2017). For obvious reasons, no work to date has measured thermoneutral zones in pregnant or lactating pinnipeds, as previously discussed. Grey seals themselves, as a result of their aquatic lifestyle, have thick blubber layers and short dense pelage to minimize heat loss in water without the need for increasing metabolic demands (Hall 2002). Previous work measuring thermoneutral properties of juveniles and pups have documented T_{uc} of up to 35^{o}C (Boily and Lavigne 1996), which is well above the ambient temperatures documented in this study. However, pups and juveniles have a much smaller body size and decreased blubber stores which may allow them to tolerate higher ambient temperatures. Adult ice-obligate phocids, such as harp seals, have T_{uc} values measured closer to that of 15^{o}C, and are similar in body size and shape to the adult female grey seals measured in this thesis (Gallivan and Ronald 1979; Worthy 1991; Kvadsheim et al. 1994;

Rommel et al. 1995). Metabolism is the main source of heat production within the body in mammals (Kleiber 1961). Metabolism in lactating grey seals is already up to seven times what would be predicted by mass alone (Mellish et al. 2000). Evidence of thermal stress in grey seals, or rather a responsiveness to increasing burdens of a warmer environment during lactation was observed to drive an increase in activity levels (**5.3.1**), depress background and daily energy expenditure as noted from heart rate data (**5.3.2**), and also driving modulations in time spent in a state of apnea (**6.3.3**) in the current study. Thermal environment has been shown to drive lactation trade-offs in a high montane rodent between populations acclimated to different altitudinal ranges (Sassi et al. 2017). In seals, the combination of increased metabolism, thickened blubber layer, especially at the start of lactation, and limited routes for non-metabolic thermoregulation, has likely caused a substantial shift in the thermoneutral zone during lactation towards a lower T_{uc}.

Increasing temperature means that individuals need to rely mostly on evaporative cooling. While potentially driving differences in water loss and water management in grey seals, evaporative cooling as temperatures increase places an even more important role for freshwater in thermoregulation. The ability to regulate internal temperature is tightly linked to hydration state; individuals are less able to regulate temperature when dehydrated (Kurta et al. 1989; Beauplet et al. 2003; Cain, III et al. 2006). As a result, females who are less able to maintain hydration may be less able to physiologically regulate internal core temperature and may risk hyperthermia. It is also worth noting that differences in solar radiation effects will mean that ambient temperatures measured in this study may be lower than what the seals actually feel on the skin surface (Codde et al. 2016). The highest measured temperature of 15^{o}C without the addition of information from solar radiation may actually underestimate the true thermal burden experienced by females on the colony. Grey seal mothers, and likely other lactating phocids, probably have a much lower T_{uc} than would be predicted by their mass-

specific metabolism, which may make them especially susceptible to the effects of a warming and drying climate across the northern hemisphere during such a critical period of life history (Jenkins et al. 2008; Foden and Young 2016). Some evidence in tropical Galapagos sea lions (*Zalophus californianus wollebaeki*) indicates that thermal environment heavily dictates the distribution of breeding animals, overriding many other considerations driving habitat use (Wolf et al. 2005). While the climate envelope for the distribution of all grey seals is especially wide (Hall 2002), lactating individuals will have a much narrower range of suitable habitat, both temporally and geographically, in order to give birth to and raise their young. Evaluating energy management in other pinniped species, such as ice-obligate ringed (*Pusa hispida*) or hooded seals (*Cystophora cristata*), may help to determine the specific adaptations that allow them to exploit a different thermal environment than their southerly cousins, but also to what degree these sensitive species are able to modulate their overall energy management strategies in the face of a changing climate.

7.3.4 – Male conflict and energy management

While this thesis focused exclusively on lactating females, grey seal males remaining hauled out and fasting to maintain breeding territories for extended periods of time (Boyd and Campbell 1971; Twiss et al. 1994; Bishop et al. 2015b, 2017) likely also adopt this compensation framework for the majority of their tenure on land. Grey seal males do not have the same short-term energetic demands as lactating females, but they can, at the peak of their condition, remain hauled out for several weeks at a time without feeding (Bishop et al. 2017). While measures of heart rate have not been undertaken with males due to logistical constraints, behavioural evidence suggests that males also prefer to minimize activity (Bishop et al. 2015b), and likely also background processes. Male grey seals apparently only selectively engage in escalated conflict, indicating that maintaining a compensation framework with overall lower daily energy expenditure may be the preferred mechanism for being able to extend fasting to

maximize mating opportunities (Bishop et al. 2017). Considering the constraints of a compensation framework of energy management, it is likely that mechanisms of conflict and conflict resolution in males likely arose with the fitness benefits of maintaining territories with efficient use of fasting energy reserves through the use of alternative modes of communication. Periods of extreme activity, such as would result from escalated male-male conflict, would quickly exceed the limits of a compensation framework for a fasting territorial male and shift towards a performance model, where activity-specific energy expenditure quickly outpaces energy used in background processes (Halsey et al. 2019). While briefly using a performance model of energy management for territorial disputes and mating opportunities, the presence of several relatively low energy behaviours, like open-mouth threats and body slaps, indicates that selection likely favours alternative energy savings routes of conflict resolution (Bishop et al. 2014, 2015a).

Other unusual behaviours in male grey seals may also be explained in the context of these energy management frameworks. Cannibalism is a rarely noted behaviour in male grey seals where males were seen to kill and consume recently weaned seal pups (Bishop et al. 2016). This behaviour is often documented towards the later end of the breeding season where males are at their poorest condition (Bishop et al. 2016; Brownlow et al. 2016; van Neer et al. 2019). Infanticide and cannibalism have been documented in wide variety of terrestrial mammals with varying hypotheses about its selective advantage, both social and energetic (Van Schaik and Kappeler 1997; Ebensperger 1998; Boyko and Marshall 2009; Borries et al. 2011). It is difficult to comment on the specific selective social advantages for this behaviour in that grey seal males are polygynous and cannot determine parentage of adjacent offspring, even when site faithful between years (Twiss et al. 2006; Bishop et al. 2016). While consuming fat-rich weaned pups certainly confers some energetic reward, cannibalistic behaviour also confers a high risk for zoonotic disease transmission (Ebensperger 1998; Forbes

2000). A strong limit in compensation energy management between balancing energy partitions for background metabolism and activity, noted for female grey seals in **Chapter 5**, likely represent a theoretical threshold where males (and probably females) may be reaching terminal (type-III) starvation and must resort to extreme measures to make up their energy debt (Mellish and Iverson 2001; Rosen and Trites 2002; McCue 2010). Cannibalistic male grey seals may have shifted their overall relationship towards an independent model of energy management (as would be indicated by a positive relationship between background energy expenditure and activity), or may have simply reached the end of preferential body reserves and are no longer able to maintain compensation management.

7.4 – Personality differences and pace-of-life

7.4.1 – Ontogeny, evolution, and maintenance of stress-coping styles

Personality differences, here expressed through inherent and repeatable differences in stress-coping styles, were found to be important drivers of almost all measured aspects of behaviour and physiology explored in this thesis, illustrating very distinct trade-offs in time and energy usage over lactation. Stress-coping styles, as previously discussed, represent a continuum of behavioural and physiological characteristics that indicate how an individual responds to different inputs of their environment (Koolhaas et al. 1999, 2010, 2011; Coppens et al. 2010). Here, stress-coping styles in adult female grey seals were defined as their median resting root mean square of successive differences (rMSSD) in heart rate across the lactation period (Marchant-Forde et al. 2004). Previous work has demonstrated that rMSSD represents a robust metric for stress-coping styles across a variety of species (Reefmann et al. 2009; Schmidt et al. 2010; Briefer et al. 2015; Grandi and Ishida 2015), in addition to female grey seals (Twiss et al. *in review*). Female grey seals who were classed as proactive (typically defined as bold and less responsive to environmental cues), spent significantly more time Alert and less time Resting (**Chapter 4**), had higher background energy expenditures, overall higher

daily energy expenditures, and a greater rate of mass loss than those classed as reactive (**Chapter 5**). Female pro-reactivity has also been documented in relation to pup growth rates, where proactive females often had higher pup growth rates, but where reactive females were much more variable (Twiss et al. 2012a). While proactive individuals in the current study were apparently more successful in efficiently transferring energy to their young, several reactive females actually outperformed their proactive counterparts. Some evidence in roe deer (*Capreolus capreolus*) suggests that survival advantages associated with the mother's stress-coping style for dependent young is heavily contingent on the environmental context; reactive mothers had higher offspring survival rates in closed habitats, while offspring of proactive mothers performed better in open habitats (Monestier et al. 2015). This lends evidence that given optimal environmental and density conditions for reactive grey seal females, which spend less time Alert and more time Resting and potentially conserving resources, may actually perform better in short-term fitness and transfer mass more efficiently than proactive females.

How these stress-coping styles arise is still largely unknown, but several clues exist as to how they develop in a population. Artificial selection for behavioural traits in model species suggest that stress-coping styles have a strong genetic underpinning (Koolhaas et al. 1999, 2010; Groothuis and Carere 2005). Some evidence also supports that personality traits associated with stress-coping styles are also heritable along matrilines of spotted hyena (*Crocuta crocuta*), though the effect was fairly small (Yoshida et al. 2016). Aggressive behaviour, linked to proactive behavioural types (Koolhaas et al. 1999), in recently weaned grey seals was also heavily tied to the rearing environment and maternal behaviour during the neonatal period, where pups with highly aggressive mothers in dense areas of a colony were also found to be more aggressive and reactive (Robinson et al. 2016, 2017). These effects of rearing environment and social stress have also been found in other species (de Jong et al. 2000; Goymann et al. 2001; Blas et al. 2007). Behaviour and development in these same grey

seals has already been linked to differences in hormone profiles in recently weaned pups and their mothers, where high levels of oxytocin resulted in more prosocial behaviour (Robinson et al. 2015, 2016, 2017). One study also noted in hyenas that external pressures, such as human disturbance, can also be important drivers of personality development (Greenberg and Holekamp 2017). Evidence in laboratory experiments points to a combination of maternal behaviour, maternal hormones, prenatal trauma, and early life experiences dictating the personality development of offspring (Curley and Branchi 2013; Groothuis and Maestripieri 2013). The ontogeny of stress-coping styles in grey seals, as a result, is likely a result of both genetic disposition and early environment that dictate the subsequent phenotypic expression of these behavioural and energetic traits.

There has been debate as to whether physiological phenotypes predicts behaviour or whether correlated behavioural selection instead drives physiological adjustments (Dochtermann and Jenkins 2007; Rosen et al. 2007; Binder et al. 2016; Debecker et al. 2016). While this topic is still being explored in the literature, one study found that personality differences directionally drive physiological adjustments over time, rather than physiology predicting behaviour (Bijleveld et al. 2014). Specifically, Bijleveld et al. (2014) found that personality differences in relation to exploratory behaviour did not change with experimental manipulations of body mass and gizzard size in red knots (*Calidris canutus*) in a wild context. Proactive individuals, often exhibiting boldness and increased exploratory behaviour, are thought to require larger or more efficient physiological machinery and pathways in order to afford the high energy lifestyle that these behavioural traits require (Careau et al. 2010). Beyond short-term individual fitness measured as reproductive output as examined in this thesis, personality was found to drive energy management and usage over time (**Chapter 5**). Rather than minimizing background energy expenditure as theory would predict in order to maintain a high energy lifestyle (Coppens et al. 2010; Réale et al. 2010; Careau and Garland Jr.

2012; Debecker et al. 2016; Careau 2017), proactive female grey seals appear to instead favour maintaining a compensation framework of energy management during lactation by simply raising the overall daily energy budget above that of reactive females. It is thought that state-dependent metabolism coevolved with personality and life-history (Dingemanse and Réale 2013; Sih et al. 2015). While putting more energy into a pup certainly translates to higher survival (e.g. Pomeroy et al. 1999; Festa-Bianchet et al. 2000; McMahon et al. 2000; Hall et al. 2002; Maniscalco 2014; Shuert et al. 2015a), how higher energy throughput of proactivity benefits females directly in terms of this compensation framework has been largely unevaluated, and it is unknown whether this high energy expenditure is maintained throughout other periods of life history. As pup personality was not evaluated in this thesis, it is unclear how maternal personality may effect offspring stress-coping style ontogeny. Given that this thesis was only able to evaluate short-term reproductive output, longitudinal studies incorporating the approaches presented in this thesis may reveal more about the overall development and flexibility of these personality differences in this long-lived carnivore.

Although several studies show variation in personality and stress-coping styles (Coleman and Wilson 1998; McGhee and Travis 2010; Yoshida et al. 2016; Huber et al. 2017), few provide evidence of how this diversity is maintained, despite the obvious trade-offs in energetic costs and fitness. Selection likely acts on maintaining the plastic components of these personality differences (Dingemanse and Réale 2013), and population-level mixtures of stress-coping styles appear to vary as a function of density and resource stability, where a stable mixture of proactive and reactive individuals were most successful when food sources are stable or abundant and intraspecific density is high (Dingemanse et al. 2004; Blas et al. 2007). Boldness is a hallmark for proactivity (Careau et al. 2010). Bold juveniles of several species are found to disperse further than those who are shyer, facilitating genetic mixing within existing populations as well as the establishment of new territories as they are better

able to exploit new resources or disturbed habitats, though often at the risk of increased predation and mortality (Raum-Suryan et al. 2004; Závorka et al. 2016; Spiegel et al. 2017); shy individuals may choose to remain close to their rearing environment and likely provide stability to existing populations and may have higher long-term survival. Differences in personality traits were found to result in differential survival for individuals who were used in a reintroduction program in that bold individuals were found to survive better upon initial establishment and produce young (Bremner-Harrison et al. 2004). The following generations from these initial bold colonizers, however, were found to have equal mixes of these behavioural types (Bremner-Harrison *pers. comm.*). Given that grey seals are iteroparous, directional selection for certain personality traits is likely lessened as fitness consequences, while only measured over two seasons in **Chapter 5**, are actually balanced across several years or even decades of reproductive effort (Desprez et al. 2018). Iteroparous species as a result create overlapping generations where temporal fluctuations in phenotype-environment matching maintain these genetic polymorphisms (Wolf et al. 2013). Longitudinal studies of pinniped species, while not incorporating measures of personality, found that females differentially invest time and energetic resources depending on the environmental conditions (McMahon et al. 2016; Desprez et al. 2018). Proactive females who are fixed in behavioural patterns and high energy output likely perform better when environmental conditions match their phenotype as they apparently are able to put greater energy into their offspring through higher MT_{eff} and energy output. Reactive females, on the other hand, may perform better on average across variable environmental conditions as both their behaviour and energy usage strategies remain flexible; reactives may be able to match a greater variety of environmental conditions, while not investing quite as much as proactive individuals overall in years of poor phenotype-environment matching. Skip breeding is a common feature of iteroparous species, where breeding cycles may be missed in times of suboptimal conditions (Pomeroy et al. 1999;

Stephens et al. 2009; Desprez et al. 2018). It is unclear if certain stress-coping styles are forced into skipping more often than others to recover from high energy expenditure in certain years, but these differences in stress-coping styles may result in very little difference in life-time reproductive output between individuals in a population, but this should be investigated further. If not incorporated into long-term analyses of fitness, these differences are likely lost at the population-level and soaked up in the overall variability of reproductive output in a given season. Phenotype-environment matching between years of variable environmental conditions likely maintains a mixture of pro-reactivity among grey seals measured here.

7.4.2 – Consequences of personality and stress management

Grey seals have been shown to benefit from being bold at an earlier age in reproduction (Bubac et al. 2018). Energy reserves are considered a labile state of an individual and can be associated with different feedback loops associated with age, learning, and resource acquisition (Stamps 2007; Wolf et al. 2013). Examining the relationship between background energy and activity energy expenditures in these grey seal females in **Chapter 5**, proactive females (higher background energy) appear to have more scope for plasticity in altering background energy, while still maintaining a compensation energy management strategy (see **Figure 5.7**). In other words, individuals who start with higher background energy (higher intercept) should be more able to adjust their metabolic output with changing demands of activity (slope) over time before reaching their minimum background threshold. Those with lower background energy (low intercept) may be less able to physiologically adjust to higher activity energy demands, without the risk of lapsing into an independent or performance model of energy management and may burn through limited energy reserves much quicker (Careau and Garland Jr. 2012; Halsey et al. 2019). Differences in plasticity of energy partitions has been demonstrated to be important for maintaining survival across females with varying social status in deer (Turbill et al. 2013). This suggests that individuals who are bold and proactive may benefit from being

plastic in metabolism and other background physiological processes in order to mitigate survival costs associated with higher energy output, despite being relatively fixed in behavioural patterns. However, if proactive individuals are unable to be sufficiently plastic in trading-off energy between background processes and activity, an individual may perform poorly in subsequent breeding attempts (Pomeroy et al. 1999; McMahon et al. 2016; Desprez et al. 2018) and risks lapsing into suboptimal energy usage and management strategies. How behaviour and stress-coping styles develop and how they may or may not remain consistent over the lifetime of an individual may reveal more as to how individuals benefit from physiological plasticity.

While this thesis has only considered a small part of a female grey seal's life history, many of the behavioural and physiological differences across stress-coping styles likely have effects on aspects of life beyond lactation. Outside of the breeding season, many female grey seals are pregnant and, following the completion of the moult and implantation, must feed on enough fish in order to support themselves and their growing foetus in addition to acquiring enough body reserves to complete the subsequent breeding season. Adult grey seals generally make fairly small geographic movements between breeding seasons, preferring to feed on the continental shelf and shelf break for prey before returning to the same rookery to breed each year (Hall 2002). The high energy lifestyle of proactive female grey seals may afford individuals the ability to dive to greater depths and may require them to forage underwater for longer periods, potentially giving proactive females a much wider range of the marine environment to exploit for food (**Chapter 5**). While this thesis could only investigate a very small portion of endogenous oxygen stores (haematocrit, **Chapter 6**), having a higher daily energy expenditure may mean that proactive individuals have a much higher aerobic dive limit than reactive individuals and as a result may also be able to spend extended time at sea before requiring time to recover. Individual differences in repeatable feeding strategies have already

been demonstrated in income breeding pinnipeds where individual females exploited spatially explicit sections of the marine environment for foraging (Beck et al. 2003; McDonald et al. 2009; Arthur et al. 2016). Sex-specific segregation of foraging habitats have also been demonstrated in a number of diving mammals, likely due to sexual size dimorphism across many pinnipeds as well as sex-specific differences in energy requirements (Breed et al. 2006; Carter et al. 2017; Bishop et al. 2018). Differing stress-coping styles and the associated differences in metabolic capacity therein may result in differential use of the marine environment, potentially resulting in resource partitioning during foraging bouts. If this is the case, personality differences in stress-coping styles may also be driven and maintained by antagonistic selection on foraging habits (Dingemanse and Réale 2013). Future work should consider these differences in stress-coping styles when tracking long-term patterns of at-sea behaviour as well as patterns of interactions and ecosystem-scale models of energy landscapes.

7.4.3 – Evidence of pace-of-life syndromes in grey seals

This discussion of personality differences ultimately weaves together into the idea of pace-of-life syndromes. Pace-of-life syndromes are an extension of life-history theory, where trade-offs in energy usage, behavioural strategies, and physiological differences coevolve and lead to vastly different individual- and population-level fitness strategies over the life time of an individual within a population (Debecker et al. 2016). Examples of this pace-of-life syndrome were demonstrated in a wide variety of taxa (Careau et al. 2010; Réale et al. 2010; Binder et al. 2016; Závorka et al. 2016; Grémillet et al. 2018; Guenther 2018), including several species of reptiles where correlated trade-offs in behaviour and physiology demonstrated two unique combinations of syndromes that result in equally high survival within the same species (Le Galliard et al. 2013; Goulet et al. 2017). While most discussion of pace-of-life focuses on differences in behaviour and life-history between closely related species, there is increasing evidence that these differences exist and are maintained within species and

may differ across populations (Réale et al. 2010). While this thesis was only able to track behavioural and physiological traits over a few seasons of effort, several arguments can be made from the results of this thesis for the existence of pace-of-life syndromes in grey seals. Despite the relatively small sample size of individuals used in this thesis, results presented here seem to indicate that two distinct pace-of-life syndromes exist in female grey seals. The first syndrome (henceforth referred to as 'dynamic' syndrome) is defined as females with low median resting heart rate variability (proactivity) associated with chronic adaptations to stress management that correlate with higher than average effort spent in vigilance-type behaviours and a higher metabolism and daily energy expenditure resulting in overall higher fitness in the short-term. The second syndrome (henceforth referred to as 'responsive' syndrome) includes females defined by high median resting heart rate variability (reactivity), while being overall more variable in behaviour and energy than the dynamic syndrome, is heavily correlated with decreased overall vigilance and a lower metabolism and daily energy expenditure and as a result were more variable in fitness measures in the short term. There was, however, no apparent effect of these syndromes on time spent in Presenting/Nursing effort, despite noted differences in short-term fitness outcomes.

The most recent dimension of pace-of-life syndromes to be described regards how thermal physiology may also correlate to these aspects of behaviour, stress responses, and energy usage. Some evidence in skinks (*Lampropholis delicata*) suggest that behavioural aspects pace-of-life are also predicted by thermal physiology (Goulet et al. 2017). Similar evidence was found with the physiology of lactation and offspring size in rodents (Sassi et al. 2017). These are supported by the observed variability found in this thesis with regards to behavioural thermal tolerance, water balance, and apnea, though the sample sizes were not large enough to directly evaluate correlations to the behavioural syndromes, dynamic vs. responsive, identified here. This thesis is the first of its kind to identify potential pace-of-life

syndromes in a wild, large mammal, though results presented here are preliminary. What remains to be seen, however, is how dynamic and responsive pace-of-life syndromes may differ in terms of various other life-history events. More specifically, it is unclear if these pace-of-life syndromes differ in initial growth rates, time to maturation, and ultimately if there is a significant difference in survival rates and lifetime reproductive output. Individuals exhibiting the responsive pace-of-life syndrome may develop more slowly from juvenile age classes and as a result may live longer than dynamic pace-of-life females. Only combining this framework over a longitudinal study will indicate whether these behavioural syndromes have appreciable differences in long-term life-history trade-offs, but it is clearly something that pinniped demographers should consider.

7.5 – Conclusions

In conclusion, this thesis has provided new tools for exploring behaviour and physiology with minimal disturbance to lactating grey seals. It has also presented extensive evidence for energy management strategies in a wild pinniped, a first of its kind, and how this allows female grey seals to maintain a high energy and contracted lactation period and has potentially identified two distinct pace-of-life syndromes. It also highlights that previous assumptions about thermal capabilities and tolerance of grey seals may not be correct; several lines of evidence here suggest that lactating grey seals likely have a shifted thermoneutral zone and are unable to tolerate higher temperatures. This thesis has also presented extensive and novel evidence for the effect that personality differences, documented here as stress-coping styles associated with measures of resting heart rate variability, have profound effects on the expression of behaviour, metabolic processes, energy expenditure, and short-term fitness outcomes. Many of these factors and analyses represent what is often included as the left-over variance associated with metabolism, behaviour, and fitness (Careau et al. 2008). If there is one key point to take away from this thesis, it is that these differences, while minute in the

scope of evolutionary constraints on the overall energy management and pace-of-life in organisms, may be among the most important drivers for the success and survival of populations over geologic scales. Maintaining this variability in personality and energy management strategies keeps a species robust to environmental variability, maintains the ability to expand or relocate geographically, and ultimately facilitates long-term survival.

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Appendix – Chapter 3

A3.1 – Random forest model error

The random forest algorithm was used to classify a variety of behaviours at various resolutions (see BCS, **Table 2.2**) for lactating grey seals using 500 trees. Random forests offer a great number of iterations (number of trees) and several layers of randomness in order to build a robust and powerful tool for classification of new data, with minimal overfitting. These homogeneous splits, referred to as nodes, are continuously subdivided until there is no longer a decrease in the Gini impurity index, *G* (or in this case, it will approach zero as a single behaviour is included in the node). Random forest fits many of these classification trees to a data set, combining predictions from all trees to classify new data (Breiman 1999; Cutler et al. 2007; Fehlmann et al. 2017). Briefly, a training dataset is sampled randomly with replacement, where the model grows one tree to classify the observations into different classes, or behaviours, by hierarchical decision making down each node (Breiman 1999; Cutler et al. 2007). This algorithm utilizes bootstrapped samples from the original dataset to grow each individual tree, using a random selection of predictor variables, or in this case accelerometry features (**3.2.1**), to partition the data. For this machine learning algorithm, the data were again split into a 75/25% training and testing set and grew 500 trees using the 'randomForest' package in R (Liaw and Wiener 2002). Out-of-bag observations, or rather those predictor variable observations not included in each bootstrapped sample, are then used to calculate model accuracies and error rates and then averaged across all trees (OOB). For each of the four BCS levels fitted with random forests, an error plot was generated using these out-of-bag observations and subsequent error rates. Each of the following plots (**Figure A3.1a-d**) illustrates the overall error rate, averaged across all behaviours in purple, while all other behaviours nested within each BCS denoted in other colours.

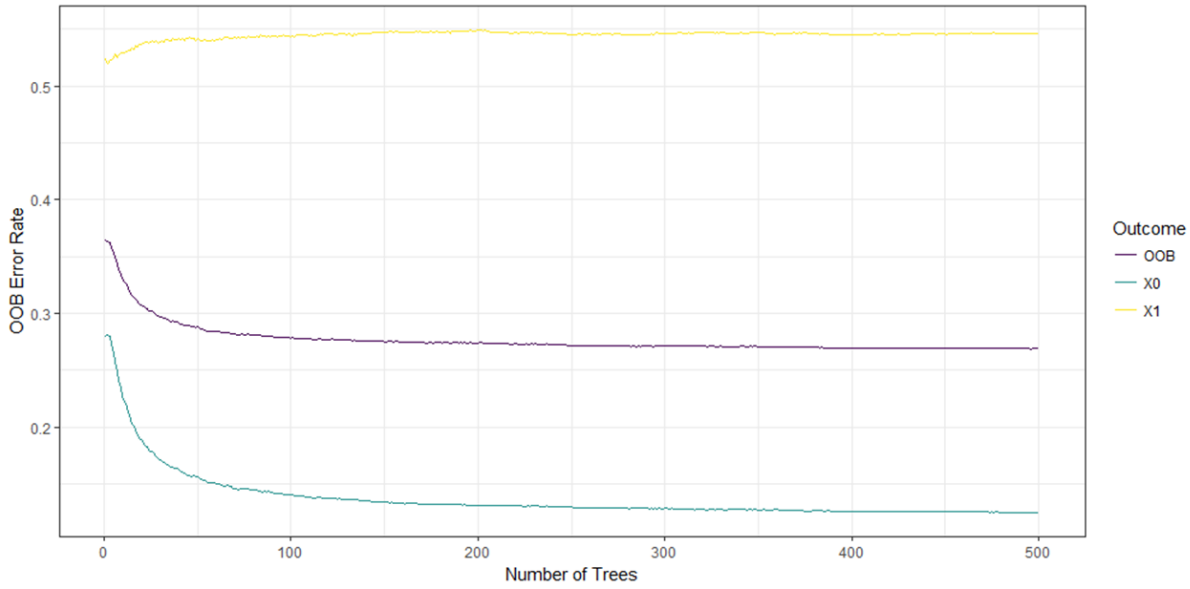

Figure A3.1a: Error plot from random forest models predicting BCS01 for 2015 head-mounted accelerometers for two behavioural states across 500 trees. Out-of-bag error estimates across number of trees shown as dark purple line (OOB). Behaviours here include inactive (X0) and active (X1).

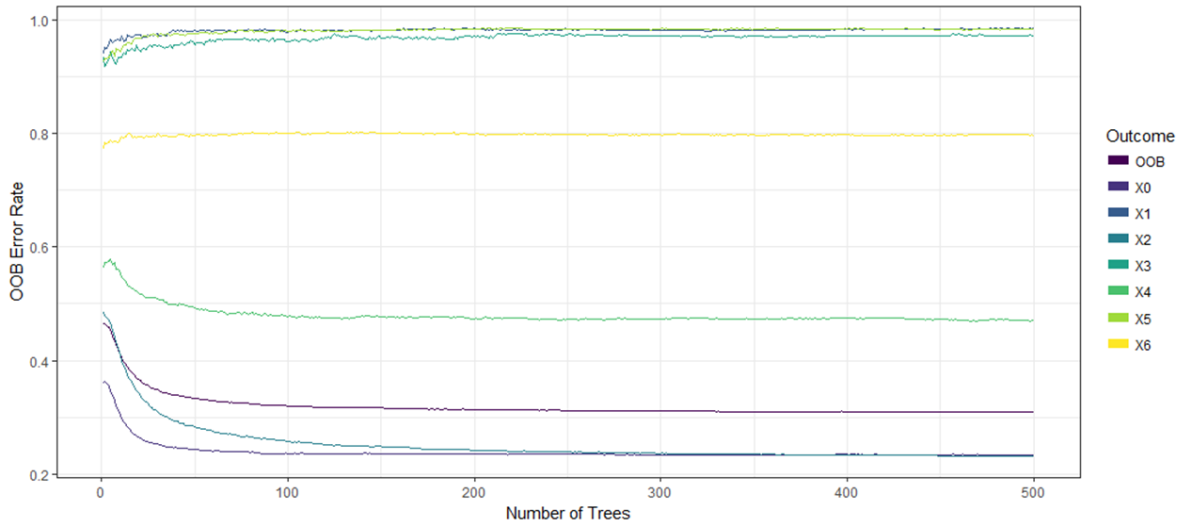

Figure A3.1b: Error plot from random forest models predicting BCScoa for 2015 head-mounted accelerometers for 7 behavioural states across 500 trees. Out-of-bag error estimates across number of trees shown dark purple line (OOB). Behaviours here include resting (X0), social/aggressive (X1), alert (X2), other (X3), presenting/nursing (X4), locomotion (X5), and comfort movements (X6).

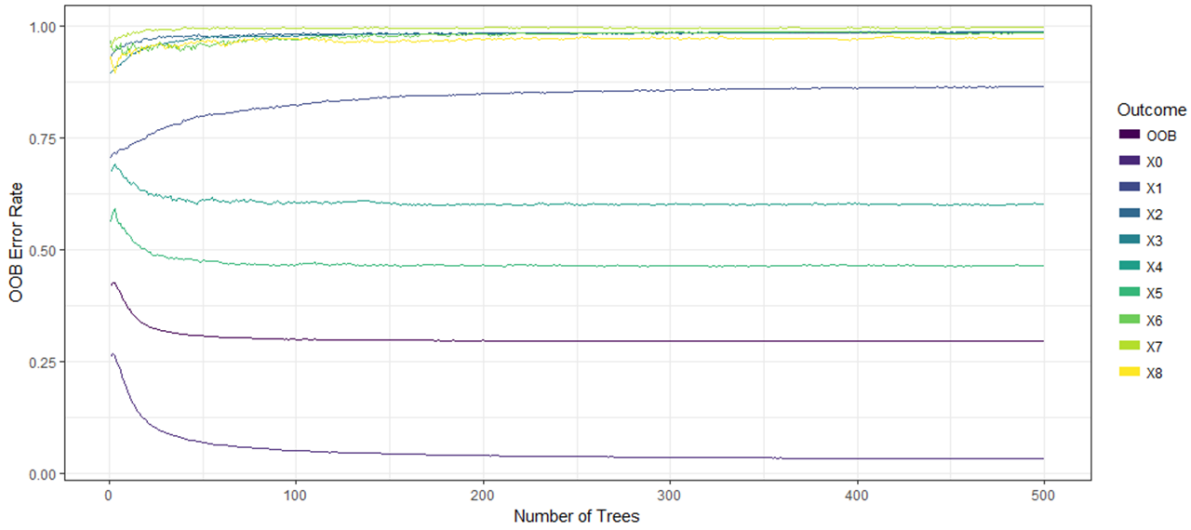

Figure A3.1c: Error plot from random forest models predicting BCSmed for 2015 head-mounted accelerometers for 9 behavioural states across 500 trees. Out-of-bag error estimates across number of trees shown as solid black line (OOB). Behaviours include resting (X0), alert (X1), locomotion (X2), comfort movement (X3), flippering pup (X4), nursing and presenting (X5), male interaction (X6), female interaction (X7), and other (X8).

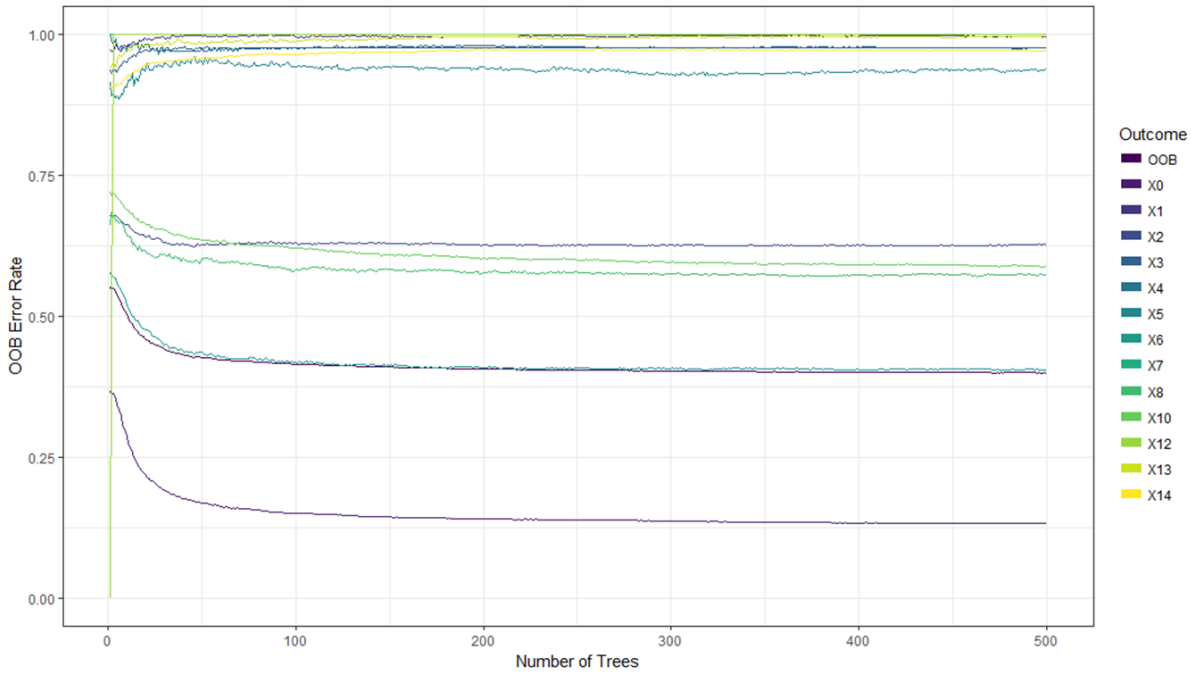

Figure A3.1d: Error plot from random forest models predicting BCSmed for 2015 head-mounted accelerometers for 13 behavioural states across 500 trees. Out-of-bag error estimates across number of trees shown as dark purple line (OOB). Behaviours here include resting (X0), social and threat behaviours for females (X1), and males (X2), and locomotion (X3), female-pup interaction (X4), other (X5), presenting/nursing (X6), chasing and fleeing (X7), flippering pup (X8), alert (X10), flippering the ground (X12), nosing pup (X13), and comfort movement (X14).

Error plots generated in from the random forest models indicate that several behaviours were poorly classified overall, regardless of the number of trees grown (error rate approaches 1). This indicates that several behaviours are regularly confused among other categories for the larger BCS classifications, and is readily apparent in the confusion matrices presented in **Table 3.2**.

A3.2 – Full random forest variable importance

Random forests also have the advantage of allowing for the assessment of variable importance by way of subtracting the parent variable Gini index value relative to the next two subsequent Gini index values for each feature variable. The full output of variable importance (relating to those variables derived in **2.6**) for 2015 head-mounted accelerometers for 7 behavioural states in BCScoa are included in **Table A3.1**. The results presented here are an extension of those found in **Figure 3.3**, which only includes the ten most important variables ranked by mean decrease in Gini. Note that the Gini index (as derived in **Eq. 3-3**), measured as the mean decrease in Gini does not approach zero indicating that a great deal of error is still present in the preferred BCS model used here, despite being the preferred model.

Table A3.1: Full variable importance table for BCScoa for random forest model classifying BCScoa representing decreasing mean Gini for each feature variable. Top 10 most important feature variables plotted in **Figure 3.3**. Feature variable derivations can be found in section **2.6** and a summary of definitions in **Table 3.1**.

Rank	Feature Variables	Mean Decrease Gini
1	stZ	5718.66
2	stY	4294.02
3	Roll	4250.46
4	Pitch	4236.94
5	stX	4170.79
6	VeDBAs	3293.00
7	PSD1X	3199.15
8	PSD2X	2886.49
9	PSD1Y	2853.73
10	PSD2Y	2449.23
11	PSD2Z	2348.49
12	PSD1Z	2321.51
13	PDBAx	2293.74
14	VeDBA	2258.21
15	PDBAy	2179.34
16	jerkN	2168.01
17	PDBAz	2164.02
18	ODBA	2139.69
19	RatioZ	2054.20
20	dyX	2029.73
21	dyY	1989.90
22	RatioX	1987.79
23	RatioY	1961.44
24	dyZ	1956.89
25	Freq1Y	1584.14
26	Freq2Z	1571.95
27	Freq2Y	1451.24
28	Freq2X	1372.67
29	Freq1Z	1364.10
30	jerkX	1358.10
31	jerkY	1352.97
32	jerkZ	1335.28
33	Freq1X	1197.15

Appendix – Chapter 4

A4.1 – Removal of pretending variables from *a priori* behaviour models

Behavioural data pertaining to time spent Resting, Presenting/Nursing, and Alert were modelled as the proportion of time spent in the behaviour of interest, weighted appropriately for the total number of seconds in each day and night period from the accelerometer data. Female ID was included as a random effect in all models to account for overdispersion and individual variability in behaviour (Bolker et al. 2009). All models (**Table 4.2**) were initially ranked based on AICc and model deviance (Anderson et al. 1998) in three different groups including intrinsic factors only, extrinsic factors only, and HRV indices separately. Results from these initial model groups are included in **Tables A4.1**, **A4.2**, and **A4.3**. Top models were selected using AICc model selection methods (Burnham and Anderson 2002). Several of these initial intrinsic and extrinsic models, while differing in model parameters, failed to improve deviance with additional covariates (< 1% difference in model deviance between two models with a single additional variable). As a result, intrinsic and extrinsic variables included in these models with equal deviance were removed as they exhibited characteristics of what are referred to as 'pretending variables' (see Anderson 2008). For models predicting time spent Resting and Alert, variables including female size and weather variables were removed. Weather variables were also removed from extrinsic models predicting time spent Presenting/Nursing. Following removal of these pretending variables, those factors included within 2 delta AICc were considered to have extensive support in modelling, while those with ≤ 5 delta AICc were considered to have minor support. Models with more than 7 delta AICc were not considered in model selection methods (Burnham and Anderson 2002). Model covariates with extensive or minor support from each of the three model groups (intrinsic, extrinsic, and HRV) were combined into a final model list and competed against the null for each of the three behavioural states to identify ultimate trade-offs and are included in **Table**

4.4. Models were described by their deviance and Akaike weight, with evidence ratios, calculated as the ratio of model weight to the null model containing only pup sex and dn (Burnham and Anderson 2002; Burnham et al. 2011). All models were built using the 'glmmTMB' package, ranked using the 'bbmle' package, and covariates model averaged with the 'MuMIn' package in R (Bolker and Team 2017; Magnusson et al. 2017).

Table A4.1: Initial model ranking for (a) intrinsic variables, (b) extrinsic variables, and heart rate variability metrics (c) for GLMMs describing time spent Resting, prior to the removal of pretending variables. Model numbers refer to those defined in **Table 4.1A**.

Time spent Resting

	Model No.	*k*	Delta AICc	Weight	Deviance
(a) Intrinsic	2	8	0.0	0.267	1532.133
	7	5	0.5	0.207	1538.765
	4	7	0.7	0.186	1534.903
	8	4	1.2	0.149	1541.452
	6	6	2.4	0.081	1538.604
	1	10	3.1	0.058	1531.091
	5	7	3.9	0.039	1538.036
	3	8	5.8	0.015	1537.921
(b) Extrinsic	11	4	0.0	0.347	1541.452
	9	5	0.5	0.274	1539.895
	10	5	1.1	0.202	1540.503
	8	5	2.0	0.129	1541.405
	7	10	5.8	0.019	1535.034
	6	11	6.8	0.012	1533.943
	5	11	7.4	0.009	1534.538
	4	11	7.9	0.007	1535.024
	3	14	11.2	0.001	1532.072
	2	14	11.3	0.001	1532.231
	1	15	13.2	<0.001	1532.037
(c) Heart Rate Variability	1	5	0.0	0.522	1375.521
	6	4	2.6	0.140	1380.179
	4	5	2.8	0.130	1378.299
	2	5	3.2	0.104	1378.299
	5	5	4.6	0.053	1380.076
	3	5	4.6	0.051	1380.170

Table A4.2: Initial model ranking for (a) intrinsic variables, (b) extrinsic variables, and heart rate variability metrics (c) for GLMMs describing time spent Presenting/Nursing, prior to the removal of pretending variables. Model numbers refer to those defined in **Table 4.1B**.

Time spent Presenting/Nursing

	Model No.	*k*	Delta AICc	Weight	Deviance
(a) Intrinsic	8	4	0.0	0.539	666.336
	7	5	1.9	0.204	666.244
	6	6	3.2	0.107	665.498
	5	7	4.4	0.060	664.608
	4	7	5.2	0.039	665.472
	3	8	6.0	0.026	664.215
	2	8	6.9	0.017	665.064
	1	10	9.2	0.005	663.327
(b) Extrinsic	7	10	0.0	0.372	649.981
	6	11	1.5	0.172	649.458
	4	11	1.9	0.144	649.821
	5	11	1.9	0.144	649.822
	11	4	4.1	0.048	666.336
	3	14	5.0	0.031	646.666
	2	14	5.4	0.025	647.076
	9	5	6.0	0.018	666.222
	10	5	6.1	0.017	666.322
	8	5	6.1	0.017	666.334
	1	15	7.1	0.011	646.666
(c) Heart Rate Variability	6	4	0.0	0.27	587.326
	3	5	0.2	0.24	585.480
	4	5	1.5	0.13	586.745
	5	5	1.5	0.13	586.819
	2	5	1.6	0.12	586.933
	1	5	1.7	0.11	587.007

Table A4.3: Initial model ranking for (a) intrinsic variables, (b) extrinsic variables, and heart rate variability metrics (c) for GLMMs describing time spent Alert, prior to the removal of pretending variables. Model numbers refer to those defined in **Table 4.1C**.

Time spent Alert

	Model No.	*k*	Delta AICc	Weight	Deviance
(a) Intrinsic	4	7	0.0	0.319	1465.020
	2	8	0.2	0.291	1463.155
	7	5	2.0	0.120	1471.052
	8	4	2.0	0.115	1473.154
	6	6	2.8	0.080	1469.818
	1	10	4.2	0.040	1463.035
	5	7	5.2	0.024	1470.185
	3	8	6.8	0.011	1469.751
(b) Extrinsic	11	4	0.0	0.318	1473.154
	9	5	0.0	0.317	1471.133
	10	5	1.1	0.183	1472.228
	8	5	2.0	0.117	1473.128
	6	11	5.5	0.021	1464.303
	7	10	5.7	0.019	1466.589
	5	11	6.9	0.010	1465.705
	4	11	7.7	0.007	1466.581
	3	14	8.5	0.005	1461.068
	2	14	9.3	0.003	1461.866
	1	15	10.5	0.002	1461.029
(c) Heart Rate Variability	1	5	0.0	0.676	1311.888
	6	4	3.8	0.099	1317.758
	2	5	4.2	0.084	1316.062
	4	5	5.1	0.053	1316.990
	3	5	5.1	0.052	1317.031
	5	5	5.9	0.036	1317.755

A4.2 – Heart rate variability models

Each of the five HRV metrics, rMSSD, mean-f_H, mean.PNSI, median.LFHF, and SD1:SD2 were competed separately using AICc model selection methods. Full model rankings for these are included in **Tables A4.1c**, **A4.2c**, and **4.3c**. Extensive support (≤ 2 delta AICc) was identified for rMSSD for predicting time spent Alert and Resting. No support was identified for any HRV metric in time spent Presenting/Nursing as all variables were equal in predictive power to the null (≤ 2 delta AICc).